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STUDIES ON THE RESPIRATION OF SEA-URCHIN SPERMATOZOA

III. RESPIRATORY QUOTIENT

BY H. MOHRI AND K. HORIUCHI
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(*Received* 8 *September* 1960)

INTRODUCTION

Sea-urchin spermatozoa are spawned into sea water which contains no obvious nutrient, and accordingly the energy for their movement must be secured exclusively from intracellular reserves. Up to the present time, several investigations have been made on the metabolism of sea-urchin spermatozoa and now there seems to be little doubt that they utilize the oxidation of endogenous phospholipids as the main source of energy for motility (Rothschild & Cleland, 1952; Mohri, 1957*a*, 1959*a*). The endogenous phospholipids are first split into fatty acids and glycero-phosphorylesters by the action of phospholipase(s), the liberated fatty acids being then utilized as substrates for aerobic metabolism (Mohri, 1957*b*, 1959*a*, *c*). These facts lead us to expect that the respiratory quotient (R.Q.) of sea-urchin spermatozoa will be approximately 0·7. The R.Q. measurements so far made, however, have rather been near unity (Barron & Goldinger, 1941; Hayashi, 1946; Spikes, 1949; Mohri, 1956*). These measurements of R.Q. were made by the Warburg direct method, which is based on the assumption that the rate of respiration is not affected by the presence or absence of CO_2. This assumption, however, has not yet been shown to be justified in sea-urchin spermatozoa and, furthermore, it is known that with certain tissues the presence of CO_2 influences the O_2 uptake. Rothschild (1956*a*, *b*) recently reported that the respiration of sea-urchin spermatozoa is very sensitive to changes in the pH of the suspending medium. According to his results, a change of 0·26 units from pH 7·91 to 8·17 caused an almost twofold increase in O_2 uptake. Since sea water possesses a low buffering capacity, it is likely that there exists a considerable difference in pH between sperm suspensions with and without absorption of CO_2 if sea water is used as the suspending medium. This would cause a serious error in the measurement of R.Q. by the Warburg direct method. In the present experiments, therefore, these points were examined and an effort was made to obtain a more accurate value of R.Q. for sea-urchin spermatozoa.

MATERIAL AND METHODS

The spermatozoa of *Pseudocentrotus depressus*, *Hemicentrotus pulcherrimus* and *Anthocidaris crassispina*, in which the aerobic utilization of endogenous phospholipids has been observed, were employed as material. Semen was obtained as described by

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* In this paper the explanation of Fig. 4 (p. 78) should be corrected as follows: (1) → (3); (2) → (1); (3) → (2).

Moriwaki (1958) and sperm suspensions were made by diluting semen with sea water, or an appropriate buffer prepared in bicarbonate-free sea water.*

O_2 uptake and CO_2 output were measured in manometers of the constant-volume type, the gas phase containing air. The R.Q. was determined by two different methods, the Warburg direct method and the first method of Dickens & Šimer (1930). In the Warburg direct method, three flasks of the conventional type were used in order to make corrections for the CO_2 retention in the medium. Two ml. of sperm suspension was placed in each flask. The O_2 uptake of the suspension during the experimental period (X_{O_2}) was known from flask 1 which contained 0·2 ml. of 10% KOH in the centre well. In flasks 2 and 3, 0·2 ml. of $5\,N$-H_2SO_4 was placed in the side-arm and tipped at the end and at the start of the experimental period, respectively. The CO_2 output (X_{CO_2}) was then calculated from the following equation

$$X_{CO_2} = \left(h_2 - h_3 \frac{K_{3CO_2}}{K_{2CO_2}} - \frac{X_{O_2}}{K_{2O_2}}\right) K_{2CO_2}, \tag{1}$$

where h_2 and h_3 represent the final manometer readings of flasks 2 and 3 after the addition of acid from the side-arm. K_{2O_2} is the O_2 constant of flask 2. K_{2CO_2} and K_{3CO_2} refer to the CO_2 constant of flasks 2 and 3. The experimental period was 60 min., and temperature was 20° C. with *Pseudocentrotus* and 28° C. with *Anthocidaris*.

In the first method of Dickens & Šimer, the O_2 uptake was first determined by absorbing the evolved CO_2 in alkali, and then all the CO_2 was liberated by adding acid to both sperm suspension and alkali at the end of the experimental period. The measured CO_2 must be the sum of the initial CO_2 and the CO_2 evolved during the experimental period. To obtain the initial CO_2 another manometer was used, in which acid was added at the start of the experimental period. The flasks used were of the special type designed by Dickens & Šimer (1930), having a trough encircling the main vessel and a side-arm. Two ml. of sperm suspension was placed in the main vessel. 0·5 ml. of cold saturated $Ba(OH)_2$ was put in the trough and 0·5 ml. of $3\,N$-H_2SO_4 was placed in the side-arm. The experimental period was 30 min. with *Pseudocentrotus* and 120 min. with *Hemicentrotus*. Temperature was 20° C.

The phospholipid content was determined in the manner described by Mohri (1959*a*). Acid-hydrolysable carbohydrate was determined by measuring the reducing sugar according to the method of Nelson (1944) after hydrolysis of the tissue with $1\,N$-HCl for 5 hr. and precipitation of proteins with a mixture of $ZnSO_4$ and $Ba(OH)_2$.

The pH of the medium was measured by glass electrodes. When buffer was used, the pH of the buffer solution was adjusted to that of sea water, about 8·2 at Misaki. The sperm density was determined with a haemocytometer.

RESULTS

R.Q. *measured by Warburg direct method.* As mentioned in the Introduction, previous studies, which gave an R.Q. of sea-urchin spermatozoa near unity, were made by the Warburg direct method, without sufficient control of the pH by means of a suitable buffer. In this work, therefore, the R.Q. was first measured by the same method, with both buffered and unbuffered medium. The results summarized in Table 1 indicate that the R.Q. obtained with ordinary sea water as diluent was somewhat lower than, but not very different from, the values hitherto reported—about 0·9. On the other hand, when sea water was buffered with either 0·025 M-glycyl glycine or 0·015 M-veronal the R.Q. was about 0·7, as would be expected from the results of previous chemical analyses. The question then arose as to why such a discrepancy was observed.

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* Abbreviations such as 0·025 M-gly. gly.-sea water, which means sea water buffered with 0·025 M-glycyl glycine, will be used in the present paper.

Table 1. *Respiratory quotient of sea-urchin spermatozoa measured by the Warburg direct method. Sperm density,* $3\cdot1$–$8\cdot8 \times 10^8$ *spermatozoa/ml.*

Species	Diluent	No. of experiments	R.Q. (mean ± S.D.)
Pseudocentrotus depressus	Sea water	5	0·93 ± 0·09
	Gly. gly.-sea water, 0·025 M	5	0·69 ± 0·09
Anthocidaris crassispina	Sea water	5	0·85 ± 0·02
	Gly. gly.-sea water, 0·025 M	5	0·70 ± 0·04
	Veronal-sea water, 0·015 M	5	0·72 ± 0·03

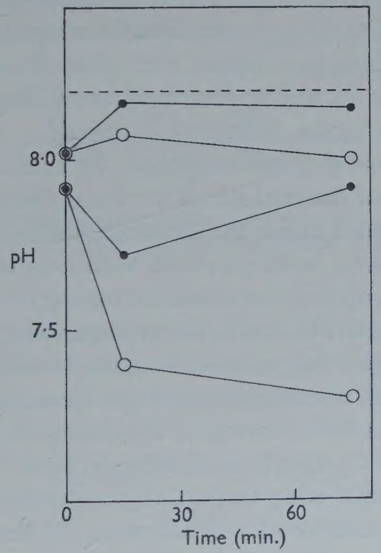

Fig. 1. Changes in pH of sperm suspensions of the sea-urchin, *Pseudocentrotus depressus*, during manometric experiments with (solid circle) and without (open circle) absorption of CO_2 by KOH. Dotted line indicates pH of ordinary sea water. Suspending medium: lower two curves, sea water; upper two curves, 0·025 M-gly. gly.-sea water. Sperm density, $5\cdot2 \times 10^8$/ml. Temp., 20° C.

Changes in pH *of sperm suspensions during manometric experiments.* Fig. 1 shows the changes in the pH of sperm suspensions during the manometric procedure with and without the absorption of CO_2 by means of KOH in the centre well. Three flasks with the same contents were used to obtain each curve. Spermatozoa were added to the suspending medium at $t = 0$ and the pH was determined. After 15 min. for temperature equilibration, the manometric readings were taken during the following 60 min., the pH being checked at the start and the end of the experimental period. When ordinary sea water was used, there occurred a sudden drop of pH from the original value, 8·20 in this case, to 7·92 immediately after the addition of spermatozoa. In the flask with KOH, however, the change in the pH of the suspension was slight thereafter, while in the flask without KOH the pH continued to drop during the equilibration period. In consequence, the respiration proceeded at different pH's during the experimental period in the flasks with and without KOH, at about 7·8 and 7·3, respectively. On the other hand, when sea water was buffered with 0·025 M-glycyl glycine, little change was observed in the pH of suspension in either case, the pH remaining not far from the original value.

As already mentioned, Rothschild (1956*b*) reported that the O_2 uptake of sea-urchin spermatozoa declines with a decrease in the pH of medium, particularly on the acid side of pH 8. If the same is true in the present material, the O_2 uptake would be less in the flask without KOH than in the flask with KOH in the case of ordinary sea water as diluent. Assuming that the metabolic sequence is unaffected by the presence and absence of CO_2, i.e. the R.Q. is the same under both conditions, $h_2 - h_3\ (K_{3\,CO_2}/K_{2\,CO_2})$, in equation (1) calculated from the obtained data would give a smaller negative value ($h_2 < h_3$ in the usual case) as compared with the value expected from the fact that there is no difference in the O_2 uptake between the two flasks. Consequently, the amount of CO_2 (X_{CO_2}) calculated from equation (1) would become larger than the true value, resulting in a higher R.Q. value. The use of buffered sea water would exclude such errors. As will be described below, however, the problem was not solved merely by considering the difference in the pH.

Effect of pH *on* O_2 *uptake of sperm suspension.* To examine the problem of whether the O_2 uptake of the present material shows a marked change with variation in the pH, an experiment was performed under the same conditions as those used by Rothschild (1956*b*). Sperm suspensions were prepared with 0·05 M-gly. gly.-sea water having different pH's, and the O_2 uptake was measured during 60 min. The results are shown in Fig. 2, in which each point indicates the average value of the pH before and after the experiments, the range being indicated by the horizontal line. In contradiction to the results obtained with *Echinus esculentus* and *Paracentrotus lividus*, the O_2 uptake of the present material was little affected by the change in the pH, although there was a tendency for the rate of O_2 uptake to decrease towards both extremes of the range used. Identical experiments using tris(hydroxymethyl)aminomethane-sea water and borate-sea water gave similar results in all cases. At least in the present material, therefore, the difference between the R.Q.'s obtained with ordinary sea water and buffered sea water could not be explained by a variation in the O_2 uptake due to the change in the pH of suspending medium, as postulated above.

In the absence of KOH, almost all the respiratory CO_2 is chemically bound in a buffer such as glycyl glycine at high pH values. According to a calculation made by Rothschild (personal communication), using a manometer with a constant of 2 and 2 ml. fluid, 90 % of the evolved CO_2 will be retained in 0·025 M-glycyl glycine, pH 8·2, at 15° C. It is necessary to ascertain whether such a situation does not have an influence on the metabolism of sea-urchin spermatozoa.

Effects of presence and absence of CO_2 *on changes in phospholipid content of spermatozoa.* In the course of studies on phospholipid metabolism of sea-urchin spermatozoa it has been noticed that diminution of phospholipids during aerobic incubation is very small when the respiratory CO_2 is not removed with KOH (Mohri, 1959*a*). As presented in Table 2, this was confirmed in the present experiments. When sperm cells were suspended in ordinary sea water, the phospholipid content diminished to about half the initial value (2·18–2·46 mg./10^{10} spermatozoa) during a 6 hr. incubation at 20° C. in the presence of KOH, whereas only about one-sixth of the phospholipids disappeared during the same incubation period in the absence of KOH. Even if 0·025 M-gly. gly.-sea water was used instead of ordinary sea water, the change in the phospholipid content was influenced by the presence or absence of KOH. In this case, however, the difference was not as marked as that observed in ordinary sea water. The difference may be less during the experimental period used in the R.Q. measurements (the first 60 min.). Under the conditions which inhibited phospholipid utilization, compensatory breakdown of carbohydrates could not be observed. The content of acid-hydrolysable carbohydrate changed, for instance, from 0·35 to 0·30 or 0·31 mg./10^{10} spermatozoa during 6 hr. incubation at 20° C. in all cases.

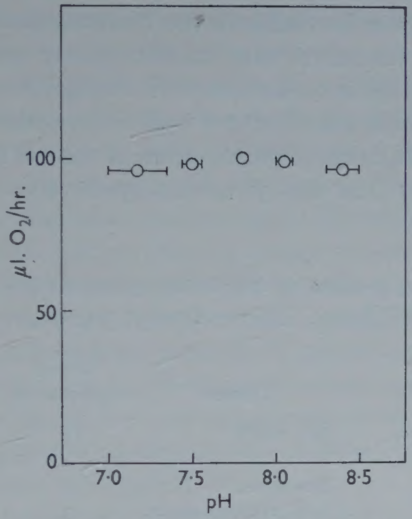

Fig. 2. Effect of pH on O_2 uptake of spermatozoa of the sea-urchin, *Pseudocentrotus depressus*. pH of sperm suspensions was fixed with 0·05 M-glycyl glycine. Sperm density, 4·5 × 10^8/ml. Temp., 20° C.

Table 2. *Disappearance of phospholipids in sea-urchin spermatozoa* (Hemicentrotus pulcherrimus) *during* 6 *hr. aerobic incubation at* 20° C., *with and without absorption of* CO_2. *All values are expressed as mg. phospholipids disappearing*/10^{10} *spermatozoa. Sperm density*, 1·09–1·2 × 10^9/ml.

Exp. no.	Sea water		Gly. gly.-sea water	
	+KOH	−KOH	+KOH	−KOH
1	0·97	0·34	1·06	0·84
2	0·88	0·45	1·05	0·84
3	1·22	0·46	1·43	1·18

Thus, assuming that the respiration of sea-urchin spermatozoa is almost entirely due to the oxidation of endogenous phospholipids, the rate of O_2 uptake would be lower in the absence than in the presence of KOH, especially when ordinary sea water is used as the medium. This would explain at least in part the above-observed discrepancy between the values for R.Q. with ordinary sea water and with buffered sea water.

R.Q. *measured by the first method of Dickens & Šimer.* In the determination of the R.Q., the first method of Dickens & Šimer is more favourable than the Warburg direct method, since the measurements of O_2 and CO_2 are both carried out on the same sperm suspension, thus excluding possible errors due to the use of two suspensions under different conditions, which is inevitable in the Warburg direct method. Although the CO_2 initially present is measured with another sperm suspension, the difference between any two suspensions is considered to be negligible. As shown in Table 3, the R.Q. obtained by this method was about 0·7 with either ordinary sea water or 0·025 M-gly. gly.-sea water. The duration of the experiment was far longer with *Hemicentrotus* than with *Pseudocentrotus* (120 and 30 min., respectively), but the results obtained were identical.

Table 3. *Respiratory quotient of sea-urchin spermatozoa measured by the first method of Dickens & Šimer. Sperm density,* $3·9–9·4 \times 10^8$ *spermatozoa/ml.*

Species	Diluent	No. of experiments	R.Q. (mean ± S.D.)
Pseudocentrotus depressus	Sea water	10	0·71 ± 0·05
Hemicentrotus pulcherrimus	Sea water	3	0·72 ± 0·02
	Gly. gly.-sea water, 0·025 M	3	0·70 ± 0·02

It has been reported that the initial high respiration following dilution of semen with sea water is sensitive to the action of sodium azide, while the subsequent steady low respiration is little affected by this agent. Conversely, 2,4-dinitrophenol or substrates such as lecithin and octanoate do not affect the initial respiration, but stimulate the rate subsequently (Mohri, 1956, 1957*a*). An experiment, undertaken to examine whether different substrates are used in the initial high and the steady low respiration, resulted in a similar R.Q. in the two cases; for example, the R.Q. measured during the first 15 min. after dilution was 0·73, while that during the 90 min. after incubation for 180 min. at 20° C. was 0·71.

DISCUSSION

The concept that the endogenous phospholipids provide the energy for maintenance of motility of sperm cells was first put forward by Lardy & Phillips (1941 *a*, *b*) in washed bull spermatozoa under aerobic conditions. Evidence in favour of this concept, however, has not been obtained in later experiments with washed boar and bull spermatozoa (Ogura, 1955; Bomstein & Steberl, 1957), in which neither a considerable decrease in the content of lipid phosphorus during aerobic storage nor oxidation of exogenous phospholipid by sperm cells could be demonstrated. Quite recently, the concept has been revived with some modifications through the work of Mann and his colleagues (Lovern, Olley, Hartree & Mann, 1957; Hartree & Mann, 1959), who found that the main component of phospholipids in ram and bull spermatozoa is not ester phospholipid but a choline-containing plasmalogen. Liberation of fatty acid from the plasmalogen necessitates a reduction in the ester linkage, but not a change in the content of lipid phosphorus. These authors measured the R.Q. of washed ram spermatozoa by the Warburg indirect method and obtained a value between 0·70 and 0·72. Thus, most of the substrates for the endogenous respiration of washed mammalian spermatozoa seem to consist of the fatty acids derived from phospholipids.

In sea-urchin spermatozoa, which when shed into sea water are in a state resembling that of washed mammalian spermatozoa, the data hitherto obtained are consistent with the above concept, except that the early determinations of the R.Q. produced a value suggesting carbohydrate metabolism. The phospholipids of sea-urchin spermatozoa, however, mainly consist of ester phospholipids, plasmalogen making up only a small portion (Hartree & Mann, 1959; Mohri, 1959*a*, *b*). The results presented above indicate that the R.Q. of about unity obtained by the Warburg direct method in ordinary sea water is a misleading one, owing to the metabolic difference between sperm suspensions with and without the absorption of respiratory CO_2. In fact, in ordinary sea water the endogenous phospholipids were metabolized at a much reduced rate in the absence, as compared with in the presence, of KOH. As there is no compensating fall in sperm carbohydrate content, O_2 uptake should fall. Judging from the results obtained in the present experiments, the cause underlying such a difference is probably the evolved CO_2 or the bicarbonate content of the medium, although a dramatic change in the O_2 uptake accompanying variation in pH was reported in other sea-urchin species by Rothschild (1956*b*).

In connexion with the respiratory dilution effect, which is defined as the increase in O_2 uptake per spermatozoon in dilute sea-urchin sperm suspensions as compared with dense ones (cf. Rothschild, 1956*b*), Runnström, Tiselius & Lindvall (1945) presented the hypothesis that the CO_2 content of dense suspensions is probably the factor which inhibits sperm motility. This hypothesis has not been taken into consideration, because in the manometric experiments in which the respiratory dilution effect is observed, the evolved CO_2 is constantly removed by KOH (Rothschild, 1948). The present results, however, seem to agree with this old hypothesis, although direct evidence is still lacking.

On the other hand, the R.Q. values, measured either by the Warburg direct method in buffered sea water or by the first method of Dickens & Šimer, were close to 0·7, a value consistent with the results of early studies. In buffered sea water, there was no marked difference in the utilization of endogenous phospholipids with and without the absorption of CO_2. Strictly speaking, these measurements, especially the latter, were performed under rather special conditions, in which almost all the CO_2 was removed and the bicarbonate content of medium also reduced, being thus somewhat different from the natural circumstances. The experiments in which the utilization of phospholipids was clearly demonstrated were also done under such conditions (Rothschild & Cleland, 1952; Mohri, 1957*a*, 1959*a*). Although measurements using the indirect method are desirable in this respect, it cannot be said that the experimental conditions in this method are the same as the natural ones.

SUMMARY

1. The respiratory quotient of sea-urchin spermatozoa has been determined with *Pseudocentrotus depressus*, *Anthocidaris crassispina* and *Hemicentrotus pulcherrimus*.

2. The R.Q. of sea-urchin spermatozoa, measured by the Warburg direct method, has been reported to be near unity. This was also the case with the present material when the suspending medium was sea water, the R.Q. being 0·8–1·0. It was found, however, that the pH of sperm suspensions was markedly different in the presence and absence of alkali to absorb CO_2.

3. When the pH of the suspension was fixed by such buffers as 0·025 M-glycyl glycine, the R.Q. measured by the above method was about 0·7. This is in accord with the results of earlier metabolic studies, which indicated that endogenous phospholipids are the main substrates for the respiration of sea-urchin spermatozoa.

4. The O_2 uptake of the present material, however, was found to be little affected by variation in pH. The difference in the R.Q. values obtained with ordinary sea water and buffered sea water, therefore, cannot be explained in terms of pH.

5. When the spermatozoa were suspended in ordinary sea water, the utilization of endogenous phospholipids was much reduced in the absence of alkali, while in buffered sea water the change in phospholipids was almost the same, with and without the absorption of CO_2.

6. Determination of the R.Q. by the first method of Dickens & Šimer, in which the O_2 uptake and the CO_2 output were measured with one and the same sperm suspension, gave a value of about 0·7 with both ordinary and buffered sea water.

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EVIDENCE FOR HUMORAL REGULATION OF DIGESTIVE SECRETION IN THE BEETLE, *TENEBRIO MOLITOR*

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INTRODUCTION

As a broad generalization it may be said that feeding results in an increase in the midgut enzyme activity of insects (the evidence for this is reviewed by Waterhouse, 1957). When due allowance was made for a spontaneous enzyme increase which occurs after the larval moult or on emergence from the pupa, this was shown to be true of *Tenebrio* (Dadd, 1956).

Information on the mechanisms whereby feeding might regulate secretion is meagre. Day & Powning (1949) adduced evidence to support the view that humoral regulation might occur in some insects. They cited *Tenebrio* in their arguments; for, having shown that in cockroaches feeding caused an increase in both enzyme activity and the rate of mitoses in the midgut epithelium, and inferring from this that increased mitosis might be taken as an index of secretory activity, they demonstrated that the injection of blood from fed into starved *Tenebrio* accelerated the rate of mitosis. Unfortunately similar cross-injection experiments with cockroaches were unsuccessful, and as no attempt was made to study directly the enzyme activity of *Tenebrio* midguts, the hypothesis that a hormone-like midgut regenerative factor might stimulate enzyme secretion remained tentative.

In choosing to study digestive secretion in *Tenebrio* one aim was to discover whether a factor in the blood, produced in response to feeding, could affect secretion as well as epithelial mitosis. Supposing that regulation of secretion always involved such a factor, its presence in the blood could also be expected during the period when endogeneous events bring about the spontaneous rise in digestive activity from zero at emergence to a high level some days later. As this spontaneous enzyme activity arises during the final differentiation of the adult (as judged by its gradual hardening and darkening from the soft, white, immediate post-emergent condition) it seemed possible that the initiation of secretory activity might be under the same general hormonal control as adult differentiation.

These considerations suggested that the following experimental approaches might yield an insight into the nature of the mechanisms controlling digestive secretion. First, the experimental situation used by Day & Powning to detect the influence of a blood factor on mitosis could be followed to discover whether enzyme activity was similarly affected. Measurements of enzyme activity were therefore compared in starved adults which had been injected with blood from either fed or starved donors. As an extension of this, it was hoped that the effect of injecting starved insects with blood taken from recently emerged adults at the height of their initial spontaneous enzyme secretion would indicate whether a similar blood factor (should such exist) was present at that time.

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A different line of approach arose from the speculation that the initial spontaneous secretion might be controlled by the hormones concerned in the events of metamorphosis and adult differentiation. To illuminate this possibility, the course of enzyme development was followed in insects whose heads had been ligatured off at appropriate times in the late pupa or shortly after emergence, it being expected that such treatment would interfere with the release of cephalically derived hormones concerned in emergence and adult differentiation. By ligaturing off the heads of pupae one day before emergence it proved possible to produce malformed adults in which the normal spontaneous development of digestive enzyme failed to occur. A few experiments were made in which the effect of injecting such recipients with blood from normal post-emergent adult donors was examined. It was hoped thereby to find whether such blood contained a factor which could rehabilitate the secretory activity suppressed by ligation.

METHODS

The work discussed here formed part of a study, the bulk of which has already been published. A detailed account of the general methods used in defining the developmental and experimental history of individual insects, the estimation of midgut protease, and the assessment of the data obtained will be found in the earlier paper (Dadd, 1956).

In blood injection experiments, adults which had been unfed for either 13 or 16 days from emergence were used as recipients. Blood drawn from starved, recently fed, or newly emerged beetles was injected into the recipients and their total midgut protease was estimated 24 hr. later when they were 14 or 17 days post-emergent. Starved donors were at least 9 days unfed from emergence. Both flour- and cellulose-fed donors were used, and were mostly known to have fed (following 14 days starvation) within the 4 hr. preceding the withdrawal of blood. Newly emerged donors were 3 or 4 days post-emergent, this being the time of greatest enzyme increase during the initial spontaneous secretory period.

The following injection technique was adopted. Without etherization, the forelegs of the donor were cut off at the coxae, when drops of clear blood welled out (under ether no such welling occurred). A further copious drop was usually obtained by snicking the membranous cuticle around the genitalia. These drops were sucked into a syringe made by inserting a glass pipette with a fine capillary termination into a convenient length of pressure tubing, one end of which could be held in the mouth and gentle suction, or blowing, applied. The volume of blood was not measured; all that could be obtained from one donor was used for each recipient. The recipient, which had meanwhile been lightly etherized, was deprived of an elytron and wing; under the binocular microscope the syringe was inserted beneath one of the posterior abdominal tergites and the blood was gently blown forward. The wound left by the removal of the wing allowed excess pressure to displace some of the recipient's own blood. Insertion of the syringe was facilitated if the tip of the capillary was so fractured as to leave one side projecting as a sharp point. The beetles were considered to have survived this operation satisfactorily if, on the following day when due for enzyme estimation, they appeared normally active. If moribund, or if on dissection the gut appeared damaged, they were discarded.

Insects used in the experiments on cephalic interference were at first merely ligatured with cotton thread at the neck. Later, and in the majority of cases, they were first ligatured loosely, then decapitated, and the ligature finally tightened, as it was found that rupturing of the cuticle behind the ligature was thereby reduced, particularly when dealing with pupae. If such rupture occurred, post-operative survival was short, presumably through loss of blood and desiccation. Both methods of interference gave similar results and are treated together. Successfully decapitated and ligatured beetles survived actively for about 14 days, and pupae operated on the day before emergence moulted (imperfectly, because of the ligature) and survived up to 10 days as active, although variously malformed, adults.

Decapitated pupae never darkened completely after moulting; if they survived longer than a day or two they usually became a light to medium reddish brown colour. Decapitated adults eventually developed various shades of darker brown, or the normal nearly black colour, depending on the time of decapitation.

When decapitated pupae were subsequently injected with blood after moulting, this was done the day following the moult. The double operation resulted in a high proportion of failures (rather more than 50%). Such doubly operated beetles were only used if they appeared active on the fifth post-emergent day when maximal enzyme activity would normally be expected. Doubly operated beetles injected with blood from pupae in mid-instar were used as controls for these experiments, it being supposed that, should a secretion-inducing factor exist, its presence would be less likely in the pupa than at any other stage. This proved to be a doubtful assumption.

RESULTS

(*a*) *Blood injection experiments*

Values for total midgut protease of starved adult *Tenebrio* injected 24 hr. previously with blood from starved, fed, or recently emerged donors are listed in Table 1. The data given for non-injected starved and fed beetles are taken from previously described experiments which were run concurrently.

For starved recipients of both ages (14 and 17 days post-emergent) the mean enzyme values of those injected with blood from donors which had recently fed were about 50% greater than the means for those injected with blood from starved donors. Increases of the same order were recorded for recipients of blood from recently emerged donors. The magnitude of the difference between starved recipients of 'starved' or 'fed' blood is thus about one-third that between normal (non-injected) starved and recently fed adults of comparable age.

These results accord well with the hypothesis that regulation of secretion might be mediated by a blood factor occurring spontaneously in adults just after emergence, or later in response to feeding. The comparatively small increases associated with the injection of active blood when compared with those occurring normally in response to feeding may be understood in terms of the probable low concentration of injected factor. A normal concentration could hardly be expected short of a complete replacement of recipient's blood by that of the donor, and it is unlikely that anything approaching this was effected by the technique used here.

It is interesting to note that, although too meagre to be more than tentative, the data for recipients injected with blood from cellulose-fed donors indicate that the occurrence of feeding, rather than the nature of the food, is of importance in activating the blood. This is consistent with the ability of inert foods (cellulose powder or water) to cause an increase in midgut protease activity of the same order as that caused by flour (Dadd, 1956).

Table 1. *Total midgut protease values of starved adult* Tenebrio *injected with blood from starved, fed, or newly emerged donors*

	Recipients starved for 14 days				Recipients starved for 17 days			Normal (non-injected) adu			
								Starved for		Recently f	
Type of blood donor	14 days starved	Flour-fed	Cellulose-fed	Newly emerged	14 days starved	Flour-fed	Newly emerged	14 days	17 days	14 days	1
Individual protease values (arbitrary comparative units)	5	12	17	8	1	12	13	5	11	23	
	7	13	21	12	3	14	13	5	—	28	
	9	14	25	16	5	15	15	9	—	33	
	10	15	25	16	7	15	17	13	—	41	
	11	15	—	17	7	16	—	14	—	42	
	12	16	—	22	9	19	—	15	—	48	
	13	17	—	28	10	20	—	15	—	48	
	14	19	—	29	11	27	—	17	—	53	
	16	19	—	33	13	—	—	17	—	—	
	16	21	—	35	16	—	—	18	—	—	
	16	22	—	—	17	—	—	19	—	—	
	16	24	—	—	21	—	—	19	—	—	
	17	25	—	—	23	—	—	20	—	—	
	18	31	—	—	—	—	—	20	—	—	
	21	48	—	—	—	—	—	21	—	—	
	22	—	—	—	—	—	—	26	—	—	
	24	—	—	—	—	—	—	30	—	—	
Means	15	21*	22	22*	11	17*	15	17	—	40	

* These means were significantly different at the 0·05 level from the corresponding means for recipients of starved bloo the titrimetric data from which the arbitrary comparative units were derived were compared by 'Student's' *t* test.

(*b*) *Decapitation experiments*

Total midgut protease values were determined for adults of various post-emergence ages which had been ligatured at the neck and, in most cases, decapitated at times shortly before or after emergence. The results are presented graphically in Fig. 1.

Decapitation of pupae a day before they moulted quite clearly suppressed the normal spontaneous secretory activity of the emergent adults almost entirely. Of twenty-eight individuals thus dealt with, twenty failed to develop measurable midgut protease. The comparatively high mean value shown for the sixth day was due to one insect having an activity of 12 units, perhaps indicative of faulty ligaturing or incomplete decapitation; the remaining seven having detectable enzyme gave values in the range 1–3 units.

Decapitation after emergence clearly delayed the development of enzyme and reduced the highest level attained to a degree dependent on the time of decapitation. When this occurred within 1 day after emergence the peak enzyme activity was about half normal and took twice as long to develop. Decapitation during the second and third days after emergence caused progressively less interference with secretory activity.

These results support the idea that a factor originating in the head might initiate and subsequently maintain secretion. Further insight into this possibility was sought by injecting newly emerged adults obtained from decapitated pupae with blood from normal post-emergent adults. These experiments were on the whole unsatisfactory, for the double operation killed over half the material, and this implies that those which survived were probably in a grossly abnormal condition. Nevertheless, the results are of considerable interest, although of tentative significance, and are set out in Table 2.

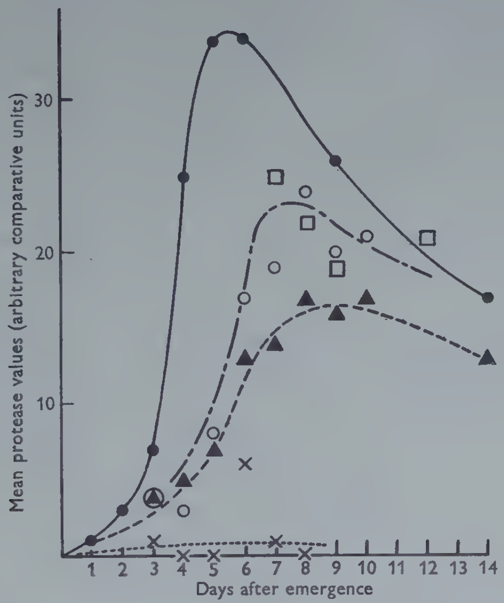

Fig. 1. Midgut protease activity of adult *Tenebrio* at various times after emergence. × · · · ·, pupae decapitated 1 day before emergence; ▲ - - - -, adults decapitated 1 day after emergence; ○—·—□, adults decapitated 2(○) and 3(□) days after emergence; ●—, normal adults. With two exceptions all points are means of from five to twelve individual estimates. The exceptions are decapitated pupae sixth day (0 and 12 units) and eighth day (0 unit).

Of thirteen non-injected individuals, nine had no midgut protease, and the remaining four only traces. The mean value for the group was 0·3 unit. The midguts of all except one of eleven individuals injected with blood from normal newly emerged adults developed protease activity. Two had moderately high values (11 and 20 units) and the mean value for the group was 3·8 units. Seven out of nine recipients of pupal blood developed low levels of protease, the mean value for the group being 1·2 units. To the extent that these meagre data have significance, they indicate that the cephalic factor concerned in the initiation and regulation of the secretory activity of the midgut is carried in the blood.

DISCUSSION

In support of their hypothesis that a blood factor might regulate digestive secretion, Day & Powning (1949) had to rely on a circumstantial argument. This shortcoming is alleviated by the evidence from adult *Tenebrio* that injection of the blood of fed, but not starved, donors caused an increase in the enzyme activity of the midguts of

Table 2. *Total midgut protease values of newly emerged adults decapitated as pupae and injected with blood from normal newly emerged adults*

Type of blood injected	None	From normal newly emerged adults	From pupae in mid-instar
Individual protease values	0	0	0
	0	1	0
	0	1	1
	0	1	1
	0	1	1
	0	1	1
	0	2	2
	0	2	2
	1	2	3
	1	11	
	1	20	
	1		
Mean value	0·3	3·8	1·2
Fraction having protease activity	4/13	10/11	7/9

starved recipients. Moreover, a similar effect followed the injection of blood from recently emerged adults, suggesting that a factor similar to that occurring in response to feeding is spontaneously present during the final stages of adult differentiation.

This last observation has a particular bearing on whether one may envisage either a special hormone concerned solely in the regulation of digestive secretion, or a humoral factor related to, dependent on, or actually one of the well-known hormones which primarily function in the regulation of growth, metamorphosis and adult maturation. The latter possibility seems more likely in view of the spontaneous development of midgut enzyme activity, while adult characters reach their full expression during the few days following emergence. The relationships between the time of decapitation and the extent of both enzyme development and post-emergence hardening and darkening support this interpretation.

Because of the well-substantiated influence that the known endocrine organs have on differentiation, and consequently on the rate of mitosis in many tissues, Day & Powning (1949) speculated that the midgut regenerative stimulating factor in the blood might originate from one of them. Their attempts to detect, histologically, heightened secretory activity in the corpora allata and cardiaca of fed cockroaches were negative, but in this connexion the early observation that digestive efficiency was reduced in decapitated *Rhodnius* is noteworthy (Wigglesworth, 1948). More recently, inhibition of midgut protease activity by removal of brain neurosecretory cells has been demonstrated in adult female *Calliphora* (Thomsen & Møller, 1959). Morphological evidence (Thomsen, 1954) suggested that the neurohormone might in this case reach the midgut via the nervi oesophagei rather than the blood, and the inhibition of enzyme activity was interpreted primarily as part of a general influence which it appears to have on protein synthesis, for removal of brain neurosecretory cells also inhibits the growth of ovaries, accessory glands, corpora allata and oenocytes (Thomsen, 1952, 1956). Evidently the effect on digestion is here only part of a complex of physiological events regulated by this hormone.

The idea that digestive secretion might be controlled by the same humoral factors whose balanced interplay governs developmental physiology commends itself for its simplicity, for the postulation of additional, specific hormones merely on the basis of the demonstration of a humoral effect is to be avoided until the possibilities of the well-defined hormones are exhausted, more particularly in view of the widening spheres of influence which extended research attributes to them. Moreover, it helps to account for the relationships which are found between feeding and digestive secretion on the one hand, and feeding, growth, metamorphosis and sexual maturation on the other. It may be envisaged that were feeding to so alter the endocrine balance as to regulate these functions in a nicely integrated way, food would most conveniently be made metabolically available for utilization at the appropriate stages of growth, development and reproduction.

This does not exclude other more specific means of digestive regulation. An arrangement of the foregoing sort is feasibly applicable to *Tenebrio* and those Orthoptera whose secretory responses are comparatively gradual (Waterhouse, 1957), but could clearly not account for the immediate secretory response to feeding found in the carnivorous beetle *Dytiscus* (Duspiva, 1939; Dadd, 1956). It is to be expected that in carnivores which take food intermittently, dependent on the vagaries of finding prey, a more precise regulation of secretion must obtain, perhaps under direct nervous control or brought about by a direct response to food or secretogogues therefrom. A mechanism of the latter type seems most likely in the mosquito *Aedes*, for whereas no evidence of a postprandial, blood-borne, humoral stimulus to secretion was found, a differential secretory response to various food substrates occurred (Fisk, 1950; Fisk & Shambaugh, 1952, 1954; Shambaugh, 1954). In these cases where food would not normally be always available, or where quite different foods are taken for different physiological purposes, it is not to be expected that digestive secretion should be closely integrated into the developmental physiology of the insect.

SUMMARY

1. Midgut protease values of starved adult *Tenebrio* injected with the blood of recently fed adults were, on average, higher than those of similar recipients of the blood of starved donors, or of untreated starved adults of the same age. A similar increase was found in the recipients of blood of newly-emerged adults.

2. Protease activity failed to develop in the midguts of adults which had been decapitated one day before emergence. If decapitation occurred after emergence, enzyme activity developed, but at a slower rate and to a lower maximal level than in the normal adult. Inhibition of the development of enzyme activity was greater the sooner decapitation followed emergence.

3. A few experiments suggested that injection of the blood of normal newly emerged adults could to some extent rehabilitate the development of enzyme inhibited by pupal decapitation.

4. These observations are discussed in relation to the hypothesis that digestive secretion may be under humoral regulation.

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A STUDY IN INSECT MULTIPARASITISM

I. HOST SELECTION AND OVIPOSITION

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INTRODUCTION

When an insect is attacked by more insect parasites than can develop upon it, competition occurs between them. Some or all of the parasite larvae are unable to complete their development and die, either through mutual starvation or by some means of physical or physiological combat. When the supernumeraries are of the same parasite species the phenomenon is known as superparasitism, and when they are of different species it is called multiparasitism.

There has, however, been confusion between these two phases of parasitism in the early literature, principally because no distinction was made between supernumerary parasitism by individuals of the same, and of different, species. The simultaneous parasitism of a host insect, first pointed out by Howard (1897), was called 'accidental secondary parasitism' by Pierce (1908), 'double parasitism' by Fiske & Thompson (1909), 'superparasitism' by Fiske (1910) and 'mixed parasitism' by Pierce (1910). The terminology was eventually standardized by Smith (1916), who defined multiple parasitism as 'that form of symbiosis where the same individual host insect is infested simultaneously with the young of two or more different species of primary parasites'; and restricted superparasitism to a superabundance of parasites of a single species attacking an individual host insect. The former term is synonymous with 'multiparasitism' of Imms (1937) and others. More recently, two further terms have been used: 'epiparasitism' by Haviland (1921, 1922) and 'coparasitism' by Kéler (1955). In his recent text-book Sweetman (1958) used the term 'multiple parasitism' to denote supernumerary parasitism regardless of the parasite species. In the present paper, however, these terms are rejected in favour of Smith's (1916) definitions which are precise and in wide usage.

The importance of multiparasitism was first brought forward by the early exponents of biological control, Pemberton & Willard (1918), who concluded that interspecific competition between opiine parasites of fruit flies in Hawaii was responsible for the decrease in numbers of the most effective parasite. This theory was criticized by Smith (1929), who showed that their conclusions were not supported by the data they presented and suggested that the observed decrease in parasitism was due to other biotic and environmental factors which became effective only when the introduced parasites became fully established. He did not deny that the introduction of several primary parasites of an insect pest might result in some multiparasitism and competition, but contended that this would be more than counterbalanced by the greater percentage parasitism achieved.

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Though the most typical condition of multiparasitism is probably that of a host attacked by a sequence of parasites (Tothill, 1922) the occasion also arises when one instar of a host is attacked by two parasites simultaneously. In this form it is strictly analogous to superparasitism which has been analysed experimentally by several authors, notably Salt (1934) and Simmonds (1943). However, despite the frequency with which the occurrence of multiparasitism is mentioned in the literature on biological control, it has received little more than cursory attention, although it is important in the study of insect behaviour and parasite populations.

Fiske (1910) recognized that the prevalence of multiparasitism 'depends entirely upon whether or not the female parasite is gifted with a prescience which will enable her to select healthy hosts for her offspring'. He came to the conclusion that the power of discrimination in insect parasites is generally absent. However, it has since been abundantly demonstrated that insect parasites are able to discriminate between parasitized and unparasitized hosts and can avoid ovipositing in the former (Salt, 1934; Ullyett, 1936; Lloyd, 1938, 1940). This experimental study of multiparasitism is therefore divided into three parts: the behaviour of the adult female parasites in host selection and oviposition; competition between their immature stages for possession of the host; and its effects on the populations of both parasite species and their host. The present paper is concerned with the first of these, and is restricted to assessing the tendency of female parasites to attack previously parasitized hosts.

MATERIAL AND METHODS

With but a few exceptions (Lloyd, 1940, 1942) almost all the published observations on multiparasitism have been made on parasitized material collected in the field. The present study has been facilitated by the selection of a parasite–host complex which can be kept in laboratory culture under controlled environmental conditions. For this purpose two species of Hymenoptera which attack the mature larvae of the moth *Ephestia sericarium* Scott (Phycitidae) were selected: *Nemeritis canescens* Grav. and *Horogenes chrysostictos* Gmelin (Ichneumonidae, Ophioninae). The biology of the former species is well known (Diamond, 1929; Beling, 1932; Ahmad, 1936), and a preliminary study of *Horogenes* has shown that it is similar to *Nemeritis* both in adult behaviour and pre-imaginal development within larvae of *Ephestia* (Fisher, 1959).

The stock of *Horogenes* was bred from a single female taken from a flour mill at Icklingham, Suffolk, and those of *Nemeritis* and *Ephestia* were subcultured from pure strains derived from single females and maintained in the Zoology Department, Cambridge, by Dr G. Salt. Their host, *Ephestia*, was reared on a mixture of equal parts of rolled oats and whole wheat flour contained in large glass jars and kept continuously at 20° C. and approximately 70% relative humidity. Routine stocks of both parasites were made up by placing 30 mature larvae of *Ephestia* into 2 lb. glass jam jars containing a layer of rolled oats of less than $\frac{1}{4}$ in. thickness with two mated female parasites and kept under the same conditions. On emergence the adult parasites were removed to cages, given water in soaked cotton-wool and food in the form of split moistened raisins. *Horogenes* is an arrhenotokous species, and after mating the females were isolated individually in glass vials until oviposition. *Nemeritis* is thelytokous, males being extremely rare and apparently sexually functionless (Thorpe, 1939).

As Lloyd (1940) has pointed out, the experimental approach to the study of multiparasitism must begin with an investigation of the oviposition behaviour of the competing adult parasites, since its occurrence depends upon the ability of the female to distinguish between parasitized and unparasitized hosts as well as on its behaviour towards those already parasitized. To test this behaviour experimentally, females of *Horogenes* and *Nemeritis* which had been isolated and fed for 24 hr. were confined in glass jars with sets of 10 larvae, five of which were already parasitized by the other species. The larvae were buried 3-4 mm. deep in a layer of rolled oats on the bottom of the jar, but no restriction was placed upon their movements; consequently there was no constant spatial relationship between parasitized and unparasitized hosts during the experiments. The parasitized hosts had previously been obtained by putting three females with 15 larvae for 4 hr. at 25° C. This gave an average of 3·6 eggs per host for *Nemeritis* and 5·1 eggs per host for *Horogenes*.

Two methods of presentation were used. In the first each jar contained five parasitized and five unparasitized hosts; in the second the parasites were presented alternately with jars containing unparasitized and parasitized hosts, each for a period of 2 hr. The host larvae were dissected after 48 hr. incubation at 25° C.; recovery of the parasite eggs was facilitated by their increase in size during this period. Mature larvae of *Ephestia* were used as hosts so as to eliminate any possibility of size discrimination in experiments designed to test the avoidance of multiparasitism.

RESULTS

Oviposition by Horogenes

In the first experiment 10 females of *Horogenes* were each exposed to 10 host larvae for a period of 2 hr. In each replicate five of the 10 hosts contained 48 hr.-old eggs of *Nemeritis* (Table 1 A). On dissection of all hosts 48 hr. later it was found that, out of all 10 replicates, *Horogenes* laid in 45 of the 100 hosts available. Of these 36 were previously unparasitized and only nine were multiparasitized.

If one assumes no discrimination by the parasite, the numbers of both groups of hosts attacked would conform approximately to a ratio of 1:1. For the 45 hosts attacked the standard error is ±3·35. Since the number of healthy hosts selected (36) exceed the number of 22·5 expected on a 1:1 ratio by more than twice the standard error, it is concluded that the parasite did not attack both groups of hosts at random. Since there was some variation in the numbers of both groups attacked in each replicate, the homogeneity of the parasites' oviposition behaviour was also tested using the χ^2 test. It was found that there was no significant difference between the results of the individual replicates ($\chi^2 = 5{\cdot}345$, $P = <0{\cdot}9$).

In a second test the females were presented with five unparasitized larvae for a period of 2 hr.; then with five parasitized larvae containing eggs of *Nemeritis* for a further 2 hr. *Horogenes* laid a total of 84 eggs in 50 unparasitized hosts and only 21 single eggs in 21 of the parasitized host larvae available ($\chi^2 = 4{\cdot}164$, $P = <0{\cdot}05$).

It was concluded from these experiments that *Horogenes* showed discrimination between unparasitized hosts and those containing eggs of another species, and that the females limit oviposition when parasitized hosts only are available to them. However, in both cases a number of hosts were multiparasitized.

Table 1. *Oviposition by* Horogenes *given a choice between unparasitized hosts and hosts parasitized by* Nemeritis

Condition of hosts available	Stage of *Nemeritis* in parasitized hosts					
	A. Egg 48 hr.		B. 1st-instar larva		C. 3rd- to 4th-instar larva	
	Hosts attacked	Eggs laid	Hosts attacked	Eggs laid	Hosts attacked	Eggs laid
Parasitized	9	11	15	15	4	5
Unparasitized	36	81	38	87	24	64
Total	45	92	53	112	28	69
Parasite–host ratio	10:100	—	10:100	—	5:50	—

The experiments were therefore extended to test the discrimination when there is a much more marked physiological difference between the parasitized and unparasitized hosts. At the end of the first instar the *Nemeritis* larva ceases to feed solely on the haemolymph of its host and begins to digest its fat body which is rapidly cytolysed as the parasite matures. Using the same methods *Horogenes* females were tested with series of hosts containing *Nemeritis* larvae in the first and third or fourth instars.

Ten females were each placed for 2 hr. with 10 hosts, five of which contained *Nemeritis* larvae in the first instar. Out of 100 hosts examined (Table 1 B), 53 were attacked. Of these, 87 eggs were laid in 38 unparasitized hosts and only 25 eggs in 15 parasitized hosts. The χ^2 test showed that the females were able to recognize the parasitized hosts and preferred to oviposit in the unparasitized ones ($\chi^2 = 9\cdot98$, $P = < 0\cdot005$). A contingency table analysis for χ^2 was applied to the results of this and succeeding experiments to test whether *Horogenes* laid proportionately fewer eggs in the already parasitized host larvae. A χ^2 of 1·7 indicated no significant difference between the proportions of eggs laid per host attacked in each group.

In a further test each of five females was given a choice between five unparasitized hosts and five containing third- or fourth-instar larvae of *Nemeritis* (Table 1 C). Subsequent dissection showed that, in all, 69 eggs were laid in 28 of the 50 hosts available, five eggs being laid in four parasitized hosts and the remaining 64 eggs in unparasitized hosts. The avoidance of the former is marked ($\chi^2 = 14\cdot285$, $P = < 0\cdot001$). There was, however, no significant difference in the proportions of eggs laid per host attacked in each group ($\chi^2 = 2\cdot25$, $P = < 0\cdot6$).

It was concluded from these tests that *Horogenes* is able to discriminate between unparasitized and parasitized hosts, and attacks a statistically significant majority of healthy hosts. However, there is no reduction in the actual numbers of eggs laid in each parasitized host when a choice is available. This contrasts with the behaviour observed in the experiment when they were given parasitized and unparasitized hosts in separate tests. There, *Horogenes* oviposited in fewer parasitized hosts and also laid fewer eggs in each. In two further experiments, therefore, the parasites were presented alternately with parasitized and unparasitized larvae. Five females were each

Table 2. *Oviposition by* Horogenes *in alternate series of unparasitized hosts and hosts parasitized by* Nemeritis

Exposure no.	No. and condition of hosts	Stage of *Nemeritis* in parasitized hosts: A. Egg 48 hr. Hosts attacked	A. Egg 48 hr. Eggs laid	B. 3rd- to 4th-instar larva Hosts attacked	B. 3rd- to 4th-instar larva Eggs laid
1	25 unparasitized	20	62	20	53
2	25 parasitized	5	13	4	6
3	25 unparasitized	18	55	15	41
4	25 parasitized	—	—	3	10
	Total	43	130	42	110
	Parasite–host ratio	5:75	—	5:100	—

enclosed with five unparasitized hosts for 2 hr. They were then transferred to host larvae containing eggs of *Nemeritis* for a further 2 hr.; finally they were allowed to oviposit in unparasitized hosts once more. The distribution of eggs in each group of hosts (Table 2A) shows a marked decrease in the number of eggs laid in already parasitized host larvae, though there is no significant difference between the numbers of eggs laid per host attacked in each group ($\chi^2 = 3{\cdot}413$, $P = 0{\cdot}10$).

In the final test of discrimination by *Horogenes*, five females were each presented alternately with five unparasitized hosts and five containing *Nemeritis* larvae in the third or fourth instar, each for a period of 2 hr. The results, shown in Table 2B, are from 25 hosts in each exposure. The reduced oviposition in hosts already containing advanced parasites is well demonstrated. As in previous experiments the χ^2 test showed that there was no heterogeneity in the distribution of eggs in the two groups of hosts ($\chi^2 = 2{\cdot}579$, $P = 0{\cdot}4$). At the end of the experiment, in exposure 4, the avoidance of oviposition in parasitized hosts broke down and egg-laying became erratic and slightly higher superparasitism occurred in the attacked hosts.

Table 3. *Oviposition by* Nemeritis *when given a choice between unparasitized hosts and hosts parasitized by* Horogenes

Condition of hosts available	Stage of *Horogenes* in parasitized hosts: A. Egg 48 hr. Hosts attacked	A. Egg 48 hr. Eggs laid	B. 1st-instar larva Hosts attacked	B. 1st-instar larva Eggs laid	C. 3rd- to 4th-instar larva Hosts attacked	C. 3rd- to 4th-instar larva Eggs laid
Parasitized	23	41	39	78	4	4
Unparasitized	39	103	39	148	23	79
Total	62	144	78	236	27	83
Parasite–host ratio	10:100	—	10:100	—	5:50	—

Oviposition by Nemeritis

The searching and oviposition behaviour of *Nemeritis* is similar to that of *Horogenes* and the same methods were used to test its behaviour in selection of hosts for oviposition.

In the first experiment, 10 females were each confined for 2 hr. with 10 host larvae, five of which contained eggs of *Horogenes* 48 hr. old. Out of 100 host larvae, 41 eggs were laid in 23 of the parasitized hosts and 103 eggs in 39 of the unparasitized hosts (Table 3A). The χ^2 test applied to the numbers of both groups of hosts attacked showed that *Nemeritis* did not discriminate between parasitized and unparasitized larvae ($\chi^2 = 3{\cdot}571$, $P = < 0{\cdot}05$), but that it laid fewer eggs in the parasitized hosts.

In the second experiment 10 *Nemeritis* females were each given a choice between five hosts containing first-instar *Horogenes* larvae and five unparasitized hosts (Table 3C). The results demonstrated that *Nemeritis* is able to distinguish between parasitized and unparasitized hosts ($\chi^2 = 12{\cdot}0$, $P = < 0{\cdot}001$) and laid proportionately fewer eggs in those parasitized hosts which it did attack ($\chi^2 = 5{\cdot}81$, $P = < 0{\cdot}05$).

Table 4. *Oviposition by* Nemeritis *in alternate series of unparasitized hosts and hosts parasitized by* Horogenes

		Stage of *Horogenes* in parasitized hosts			
		A. Egg 48 hr.		B. 3rd- to 4th-instar larva	
Exposure no.	No. and condition of hosts	Hosts attacked	Eggs laid	Hosts attacked	Eggs laid
1	25 unparasitized	24	62	17	48
2	25 parasitized	10	33	3	4
3	25 unparasitized	18	60	13	47
4	25 parasitized	8	25	5	11
	Total	60	180	38	110
	Parasite–host ratio	5:100	—	5:100	—

The last two experiments with *Nemeritis* were designed to test the oviposition behaviour of the females to alternate sets of parasitized and unparasitized hosts, presented successively for periods of 2 hr. each. In the fourth test the parasitized hosts contained 48 hr.-old eggs of *Horogenes*. Five female *Nemeritis* were each exposed to four sets of five host larvae during an 8 hr. period. The hosts in the first and third periods were unparasitized; those in the second and fourth periods contained 48 hr.-old eggs of *Horogenes*. The results shown in Table 4A are totals of oviposition by all females in the 25 host larvae from each exposure. It is apparent that *Nemeritis* attacks fewer parasitized than unparasitized hosts ($\chi^2 = 10{\cdot}933$, $P = 0{\cdot}02$), although it does not restrict the numbers of eggs laid in each host attacked ($\chi^2 = 2{\cdot}398$, $P = 0{\cdot}4$).

In the final test, this experimental series was repeated with the parasitized hosts presented in the second and fourth exposures containing *Horogenes* larvae in the third or fourth instar (Table 4B). The reduction of oviposition in the parasitized hosts was again significant ($\chi^2 = 13{\cdot}54$, $P = 0{\cdot}001$).

DISCUSSION

The experiments described here demonstrate that both *Horogenes* and *Nemeritis* can discriminate between hosts and can to some extent avoid multiparasitism. Such a power of discrimination between hosts they encounter does not imply a comparison by the parasite of the two groups of hosts available for attack since each larva is approached individually by the parasite as a prospective host. Whether an egg is laid in it or not depends upon the sum total of the responses to the stimuli the parasite receives from that host.

Nevertheless, both parasites do achieve the effect of discrimination either by not ovipositing in hosts that are already parasitized, or by laying fewer eggs in them than they do in healthy unparasitized hosts. This behaviour continues when the parasites are exposed successively to parasitized and unparasitized hosts or offered a simultaneous choice between them.

There is, however, a considerable variation in the percentage of multiparasitism achieved by both species in similar experiments. Whereas the data show that *Horogenes* can distinguish a host containing eggs or larvae of *Nemeritis* at any stage of development, and may avoid ovipositing in it, there is no statistically significant restriction of oviposition in those parasitized hosts that it does attack.

Nemeritis, on the other hand, attacks both groups of hosts more readily than does *Horogenes*. The numbers of parasitized and unparasitized hosts attacked cannot be separated statistically on experiments of choice when the parasitized hosts contain eggs or first-instar larvae. Yet there is a statistically significant reduction in the number of eggs laid in those that are already parasitized. However, if no choice is available and the parasitized and healthy hosts are presented alternately, as they are in the latter experiments, both species restrict their attack on parasitized hosts. In general, the actual numbers of eggs laid in them are also reduced, although not to a level of statistical significance.

These results are in accordance with the partial avoidance of superparasitism observed for *Nemeritis* by Simmonds (1943) and for *Angitia* (*Horogenes*) *cerophaga* Grav. by Lloyd (1940). It is clear that the distribution of eggs by these parasites is not made at random but is related to the nature of the available hosts (cf. Lloyd, 1938). The progressive reduction in the numbers of eggs laid in *Ephestia* larvae containing advancing larval stages of parasites indicates that the adult females of both species are able to perceive the decreasing suitability of such larvae as hosts.

The wholly artificial parasite–host densities of these laboratory experiments preclude their quantitative application to the extent of multiparasitism in field populations. But they do demonstrate that the avoidance of multiparasitism, even of hosts containing advanced larval stages of another species, is only partially developed. Thus multiparasitism of *Ephestia* can be expected to occur in natural populations of *Horogenes* and *Nemeritis*, and, although the extent to which it does so is dependent upon the abundance of both parasites and host, it is most probable in hosts containing eggs or larvae in the first instar.

SUMMARY

1. The oviposition behaviour of the ichneumon flies *Horogenes chrysostictos* and *Nemeritis canescens* has been examined in relation to their multiparasitism of larvae of the moth *Ephestia sericarium*.

2. *Horogenes* is able to distinguish healthy hosts from those that are parasitized by *Nemeritis*, both when given a simultaneous choice between them and when exposed to each alternately.

3. Although *Horogenes* attacks fewer parasitized than healthy hosts, it does not lay proportionately fewer eggs in a parasitized host.

4. *Nemeritis* is also able to distinguish healthy hosts from those parasitized by *Horogenes*. Though it attacks parasitized hosts more readily than does *Horogenes*, it lays fewer of its eggs in them.

5. Both species avoid ovipositing in a host containing an advanced larval parasite more frequently than in a host containing a parasite egg.

6. An *Ephestia* larva multiparasitized by *Horogenes* and *Nemeritis* may therefore contain these parasites at widely different ages, but the host selection behaviour of the adult parasites makes multiparasitism by parasite larvae of similar age more likely in field populations.

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THE WATERPROOFING MECHANISM OF ARTHROPODS

II. THE PERMEABILITY OF THE CUTICLE OF SOME AQUATIC INSECTS

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INTRODUCTION

Recent investigations into the permeability of the integument of terrestrial insects (Beament, 1958*a*, 1959) have given new information about their waterproofing mechanisms. The cuticles of such insects have a range of permeability: from less waterproof plant-eating larvae to very impermeable pupae. In order to explain the properties of the more permeable cuticles, it might be sufficient to assume merely that there is a layer of wax of appropriate thickness. However, the extreme resistance to water-loss of insects such as *Rhodnius* cannot be accounted for in this way; it is postulated that there is a special molecular configuration of the wax, which has exceptional impermeability to water. When the effect of temperature upon permeability is also considered, it appears that even the more permeable terrestrial forms may show transition phenomena, which are characteristic of organized monolayers of lipid. Such studies of the effect of temperature also indicate, through the nature of transition and the reversibility of the process, whether the lipid on the cuticle is a grease or a wax.

Although such detailed physico-chemical information can only be revealed by technically complex methods, an earlier investigation by Holdgate (1956) showed conclusively that many aquatic insects are altogether more permeable to water than their terrestrial counterparts, though some aquatic species might approach the degree of waterproofing of the more permeable terrestrial forms. The object of the present study is to investigate further the cuticular permeability of some representative aquatic insects, and to suggest underlying mechanisms.

MATERIAL AND METHODS

Since waterproofing structures are known to be very delicate and are easily damaged by handling (Beament, 1959), the insects were collected, and later transferred from one medium to another, in small nylon nets. Most experiments were carried out soon after capture in the field. The animals were killed by introducing cyanide, azide or hydrogen sulphide into the water containing them, but it was often necessary to raise the concentration of carbon dioxide in the water to the point of anaesthesia, before adding the poison, in order to prevent self-inflicted damage. As soon as death had occurred, animals were washed repeatedly in tap water, while resting on nylon gauze, and transferred to the measuring equipment after superficial water had been allowed to drain away. The equipment for determining water-loss, surface and air temperatures under controlled conditions has already been described (Beament, 1958*a*, *b*, 1959).

EVAPORATION FROM INTACT ANIMALS

At the commencement of an experiment, an animal has free moisture on its surface, so that desiccation must be continued for a time sufficient to establish equilibrium conditions in the surrounding air and a uniform gradient across the cuticle, before measurement is meaningful. With the less permeable animals, the criteria for such conditions are readily obtained: an initial high rate of evaporation, representing the removal of superficial water, falls within a very few minutes to a steady value, and the difference in temperature between the air and the cuticle surface correspondingly decreases. Only for animals as small as *Gyrinus* adults, which are also relatively waterproof, is it then necessary to take any special precautions to conserve the water reserves of the specimen during a determination of the permeability/temperature characteristic. When the cuticle is very permeable, on the other hand, high wind speeds are needed in order to ensure that conditions obtain in which evaporation is limited by the cuticle, rather than by diffusion in the air (see Beament, 1961*a*). In dry air, such circumstances then lead to the very rapid depletion of the animal's internal water reserves. In order to obtain the rates of evaporation from some of the more permeable insects which appear in Table 1, values determined in high-velocity dry air have been compared with others obtained in moving air of higher humidities; the more permeable insects moreover appear to have rather greater variation between individuals, compared with the consistent values obtained with more waterproof species. The figures quoted in Table 1 are means from both kinds of measurement. It must be borne in mind that some authors have claimed that curious effects may result from promoting high rates of evaporation in strong currents of air; Holdgate (1956) suggests that a nymph of *Anax* loses much less water when exposed to a jet of dry compressed air, than when in an unstirred desiccator. Edney (1951) believes that attempts to create high rates of desiccation can actually decrease the permeability of the cuticle of woodlice. No evidence lending decisive support to these contentions was obtained in the present series of experiments.

In Table 1 the rates of evaporation obtained at 20° C. are expressed both per animal and per unit area of surface. The latter are included primarily in order that they may be compared with corresponding measurements by Holdgate (1956) who quoted his results in this form. However, the difficulty of arriving at a meaningful figure for the evaporating surface of an insect is well known. The areas used for present calculations have been obtained through geometric considerations, and from camera lucida drawings of dissected specimens. But as these figures (and those of Holdgate, in his own estimation) are liable to an error of perhaps 50 %, only major divergences between the two investigations need receive serious consideration. Relatively close agreement has indeed been obtained for the majority of the more waterproof insects, such as the dytiscids and the anisopteran dragonflies. The rates obtained with the more permeable insects in the present study are apparently rather higher than those given by Holdgate; but, in particular, the value for *Sialis* is much closer to Holdgate's figure than to the substantially higher figure given by Shaw (1955). The divergence of the present

Table 1

Species	Rate of evap. at 20° C. in mg./unit S.D./hr.			Species	Rate of evap./animal/unit S.D./hr. at 20° C.
	Per animal	Per cm.^{2}	Per cm.^{2}*		
Tipula larva	0·95–1·1	0·9–1·2	0·72	*Dytiscus* larva	1·5–2·2
Sialis larva	0·25–0·3	0·2–0·3	0·4	*Nepa* adult	0·4–0·5
Coenagrion nymph	0·3–0·4	0·075–0·1	0·26	*Labellula* nymph	0·2–0·3
Notonecta adult	0·27	0·1–0·15	0·18	*Ephemera* nymph	1·2–1·3
Corixa adult	0·25–0·5	0·07–0·1	0·11	*Corixa* nymph	0·45
Aeshna nymph	0·14	0·013	0·055	*Ilybius* adult	0·06
			(*Anax*)	*Gyrinus* adult	0·01
Agabus adult	0·05–0·06	0·03–0·04	0·055	*Pieris* larva†	0·15
Dytiscus adult	0·12–0·2	0·01–0·015	0·019 (*Hydrobius*)		

* Data calculated from Holdgate (1956).
† From Beament (1959).

results from those of Holdgate, when more permeable insects are considered, may be explained on a number of counts. Owing to the greater efficiency of the circulation of air in the present apparatus, the conditions of the experiment may have approached more closely to the idealized state in which evaporation is limited by the membrane alone (Beament, 1961 *a*). Holdgate's figures were calculated on the assumption that the air was at 0% R.H. throughout each desiccation period, whereas the values here presented have been compensated for the rise of humidity of the air in the desiccation chamber during each period of desiccation. But further, the present values are quoted at cuticle temperature; those of Holdgate are quoted at air temperature. Because the temperature of the cuticle is lowered by evaporation, the present values might more correctly be compared with values which Holdgate would have obtained at air temperatures a few degrees above 20° C.

Finally, Holdgate (1956) observed that the pupa of *Tenebrio* at 20° C. was at least ten times as impermeable to water as the least permeable of the aquatic insects he examined. According to more recent measurements (Beament, 1959), this extremely efficiently waterproofed animal, with two wax layers, may be as much as fifty times as impermeable as terrestrial larvae, such as those of *Nematus* or *Pieris*. The more impermeable of the aquatic insects which have now been investigated are of precisely the same order of permeability as the less waterproof terrestrial forms, and there is no *a priori* reason for suggesting that they differ materially in waterproofing mechanism on this evidence alone.

THE PERMEABILITY OF DIFFERENT PARTS OF THE CUTICLE

When insects are as permeable to water as some of those under present consideration, there is a more serious objection to the expression of results in terms of a rate of evaporation per unit area of surface. If an animal is excessively impermeable to water, then the simplest hypothesis assumes that its surface is uniformly covered by lipid, and has uniform impermeability; for if any unwaterproofed areas were to exist, then the permeability of the remainder would have to be impossibly low. Indeed the krypton adsorption method for measuring the surface area of insects (Lockey, 1960) gives such high values of area as to lead to a similar difficulty. When, however, an animal has a high rate of evaporation, in relation to its order of size, the cuticle giving rise to this could equally well comprise some areas of high permeability, the remainder having an efficient covering of lipid, or it could have uniformly intermediate permeability throughout the surface. Very little indeed is known about the distribution of cuticular lipids in aquatic insects. From the effect of ligating the anal gills of mosquito larvae (Wigglesworth, 1934) it is possible to deduce that the gill cuticle must be much more permeable to water than that over the rest of the animal; Shaw (1955) found that there was not significantly less lipid on the gill surface of *Sialis* as compared with the general abdominal integument, and in order to explain the greater evaporation rate from this insect than from typical terrestrial forms, he suggested that the waxes of *Sialis* must have 'different chemical and physical properties'. He points out that such a layer of wax on the gill would not greatly resist the diffusion of oxygen, while it would still control the movement of both water and salts. In the absence of direct measurement of the comparative permeability of such wax to oxygen and to water, the larger size of the oxygen molecule must be set against the validity of such an assumption. Shaw does, however, say that the surface of the abdomen and gill cuticle does not give a polyphenol reaction with ammoniacal silver nitrate, whereas the remainder of the integumental surface does so. Since the nature of the substrate is believed to be of very great importance in relation to the formation of organized monolayers of lipid (Beament, 1945, 1958*a*, 1960), further investigation into the permeability of different parts of some of the more permeable insects which appear in Table 1 is desirable.

Table 2

Species	Rate of evap. (mg./animal/unit S.D./hr.)		Percentage area left uncovered by 'Newskin'	Percentage of original evaporation
	Intact	Gills covered in 'Newskin'		
Ephemera nymph	1·2–1·3	0·8–0·9	65	62–72
Coenagrion nymph	0·3–0·4	0·2–0·27	70	51–56
Sialis larva	0·25–0·3	0·06–0·07	50	24–30

Following the measurement of the over-all rate of water-loss from a specimen of one of the more permeable species, the gill area was painted over with a solution of 'Newskin', a proprietary solution of pyroxylin in alcoholic solvents. This material was shown, in separate experiments, to be relatively (but certainly not outstandingly) impermeable to water; it has the valuable property, however, of extremely high adhesion to cuticle surfaces and its local application does not seem materially to affect the property of the surrounding cuticle. The very greatest care was exercised during this process so that mechanical damage to the cuticle was avoided. In Table 2 the rates of evaporation from intact animals are compared with those when the gills have been so covered. Now some reduction in the rate of water-loss would result from such a treatment, even if the animal had a uniformly permeable surface; and unless the film of 'Newskin' were entirely impermeable to water, the degree of reduction would not even be proportional to the area which was covered, compared with the area left uncovered. The mere fact, as Table 2 shows, that some reduction obtained from this treatment is of little consequence; what is important is that in the cases both of the damselfly and also of *Sialis* the degree of reduction was significantly greater than would have occurred had the animal been uniformly permeable all over, and had the gill area been made completely impermeable. No explanation is possible other than the conclusion that the gills of these animals are substantially more permeable to water than the remainder of the cuticle. The experiments on mayflies are inconclusive on this point. Because neither cuticle, nor 'Newskin' films, are likely to obey Fick's Law at all closely, we are not justified in using the data in Table 2 to calculate the actual permeability of the gill cuticle. That information has been obtained in the following section.

THE EFFECT OF PERMEABILITY ON TEMPERATURE

The assessment of the actual permeabilities of different areas of the cuticle on one insect is based on the measurement of surface temperature; Beament (1958*a*) shows that the temperature of the cuticle surface is lower than that of the surrounding air by an amount proportional to the rate of evaporation proceeding from it. In exploratory experiments in which a thermocouple probe was successively applied to different points on the surface of insects suspended in a stream of dry air (the reference junction being in the air, upstream of the specimen) it was found that the cooling of some gill surfaces exceeded that of the head or thoracic cuticle by some three or four centigrade degrees. In other cases, little difference occurred between the temperatures of different areas of a specimen. The results of a series of experiments conducted in this way, in an air stream controlled to a temperature of 15° C. at a point immediately before the air reached the specimen, are reported in Table 3.

Table 3

Species	Temperature depression on thorax (centigrade degrees)	Other area tested	Temperature depression of other area (centigrade degrees)	Calculated evap. (mg./cm.²/unit S.D./hr.)	
				Thorax	Gill
Ephemera nymph	0·9–1·05	Gill	1·0–1·1	—	—
Coenagrion nymph	0·3	Gill	0·55	0·16	0·3
Sialis larva	0·15	Gill and abdomen	0·65	0·075	0·32
Dytiscus larva	1·9	Abdomen	2·0	—	—
Notonecta adult	< 0·1	Plastron region	<0·1	—	—

It is seen from Table 3 that a number of insects which have exposed gills—mayfly and zygopteran larvae, and *Sialis*—are evaporating water very readily from those gills. The damselfly and *Sialis* are, further, losing water much more rapidly from their gills than from the rest of the cuticle, for the remaining cuticle is at a much higher temperature. *Ephemera* does not show this distinction; like the larva of *Dytiscus* it appears to be equally permeable over its entire surface. The adult water-beetles, anisopteran nymphs and *Notonecta* appeared to be equally impermeable over their entire surface, as judged by the readings of the thermocouples. While such a result is not unexpected, it is worth noting that the thermocouples do not indicate any significant difference in permeability between the main, hydrophilic areas and the extremely hydrofuge plastron region of *Notonecta* despite the great difference in surface property.

As an approximation—and certainly as accurately as surface areas can be estimated—the rate of evaporation may be assumed to be *directly* proportional to the depression of the surface temperature. The total recorded evaporation rate from a specimen is made up of the sums of the products of each local evaporation rate multiplied by each local area. There is no reason why an insect should not have continuously varied permeability over its surface; but simplifying the situation through the assumption that a specimen has two levels of permeability—over the gill area and over the remaining cuticle—we can derive those permeabilities. If we divide the total rate of water-loss of a specimen in the ratio of

$$\frac{\text{area of gill} \times \text{temperature depression of gill}}{\text{area of remainder} \times \text{temperature depression of remainder}}$$

we obtain figures for the respective permeabilities of the two regions. Values obtained in this manner have been included in Table 3 for the two species where higher rates of evaporation were demonstrated from the gills. The results obtained in the following section have been treated in a similar manner.

THE EFFECT OF TEMPERATURE UPON PERMEABILITY

According to Holdgate (1956) the greater 'porosity' of the general cuticle of the aquatic insect would give rise to a permeability/temperature relationship in which permeability was very dependent on temperature. But now that we have demonstrated the differences which can occur in the permeability of two areas of one individual, the relationship between permeability and temperature must be interpreted in this light. From the measurements given in Tables 1 and 3, the aquatic insects used in this paper can be divided into three categories: (*a*) insects of uniformly low permeability, (*b*) insects of uniformly high permeability, (*c*) insects with more permeable gills. Insects of type (*a*) were expected to compare closely in property with typical terrestrial insects, and the method of measuring their permeability/temperature characteristic (Beament, 1958*a*, 1959) was applied to them without modification. The results of these experiments are represented in Fig. 1. With the addition of precautions to ensure that permeability rather than diffusion was the limiting factor (p. 278), animals in category (*b*) were similarly investigated. Despite the short time-intervals during which readings can be obtained at any one temperature in the apparatus, the water reserves of some of these very permeable insects are so rapidly depleted that some of the curves in Fig. 2 are constructed from data obtained from more than one specimen.

The recording of two independent surface temperatures simultaneously, during a permeability/temperature determination, presents a difficult technical problem. The beam of the balance on which the specimen is exposed to desiccation has a bifilar suspension; the thermocouple array (consisting of a probe junction against the cuticle and a reference junction in the air) is connected to the galvanometer through the two elements of that suspension. Thus an additional thermocouple array, to measure a second cuticle temperature, cannot be added, for there is no way of increasing the number of suspension elements. In consequence, three methods of procedure were adopted. A preliminary experiment was conducted on a number of specimens, with the probe junction either against the thorax, or against the gill-area; the ranges of temperature and of desiccation rate which were likely to be of particular interest were thus determined. By rapid manipulation, and by judicious selection of temperatures, it was then possible to carry out a determination of the permeability/temperature characteristics, with the probe against the thorax, followed by a similar determination over the whole temperature range with the probe against the gills, on one specimen, without depleting its water reserves too greatly. Correspondence of the rates of desiccation during the second run, with those obtained in the first run, acted as a check on depletion. This experiment relies on the assumption that the cuticle is not irreversibly affected by its first experience of higher temperatures; the validity of this assumption was confirmed with the material used. Secondly, a single series of permeability measurements was made, over a selected range of temperature, but the position of the probe junction was alternated between thorax and gill for each successive reading. After a number of experiments of the preceding types, the form of the curve for total evaporation against air temperature was well established. In the third series of experiments, the balance beam was therefore clamped, and a second thermocouple array was introduced into the desiccation chamber so that both thoracic and gill temperatures could be recorded simultaneously against air temperatures, without the corresponding values of evaporation rate. It was fortunate that the material, whose properties are represented in Fig. 3, showed a relatively high degree of uniformity within a species.

Two types of waterproofing system are represented in the graphs of Fig. 1. *Dytiscus* and *Gyrinus* adults produce a curve of Z shape, similar in general form to that of the cockroach, *Periplaneta* (Beament, 1958*a*). The adults of *Agabus*, *Ilybius* and *Notonecta* produce the L-shaped curve, which is more typical of the majority of terrestrial insects so far examined (Beament, 1959). From the theories proposed in the two papers which have just been cited, we should conclude that *Dytiscus* and *Gyrinus* have a grease as their waterproofing material, whereas the others are covered by a harder wax. Greases are believed to have lower mean molecular weights than waxes, so that it is not surprising to find that the transition temperatures of *Dytiscus* and *Gyrinus* are appreciably lower than those of the others. But attention is drawn to the extremely low transition temperatures of all of these insects: that of *Dytiscus*, for example, is ten degrees lower than the corresponding value for *Periplaneta*.

There is little of note that we can deduce from the permeability/temperature characteristics of the very permeable insects. On the one hand they are so permeable to water over the entire temperature range which has been examined, and on the other the circumstances in which this high permeability has been measured differ so greatly from those in the natural environment that one cannot attempt to attribute biological significance to the effect of temperature which has been recorded.

The dissection of the over-all permeability/temperature curves for the three larvae with external gills—*Ephemera*, *Coenagrion* and *Sialis*—into the component curves for the gill area and for the remaining cuticle reveals some interesting biological information. The mayfly can be summarily dismissed; if the gills were allowed to collapse on to the surface of the animal, no difference in temperature depression could be demonstrated anywhere on the surface of the animal, but if the gills were lifted, they shrivelled rapidly and their surface temperature was then higher than that of the rest of the animal. While *Ephemera* would seem to have a cuticle rather less permeable to water than would be expected from a completely lipid-free surface, it is clear that the gill cuticle is not specially permeable to water, and, by inference, not specially permeable to oxygen either. This would agree with the observation (Thorpe, 1933) that these insects can respire adequately through the main cuticle in well-aerated water, but that the gills may become important only when the animal is in less favourable circumstances of oxygen supply or demand. That evaporation from the exposed

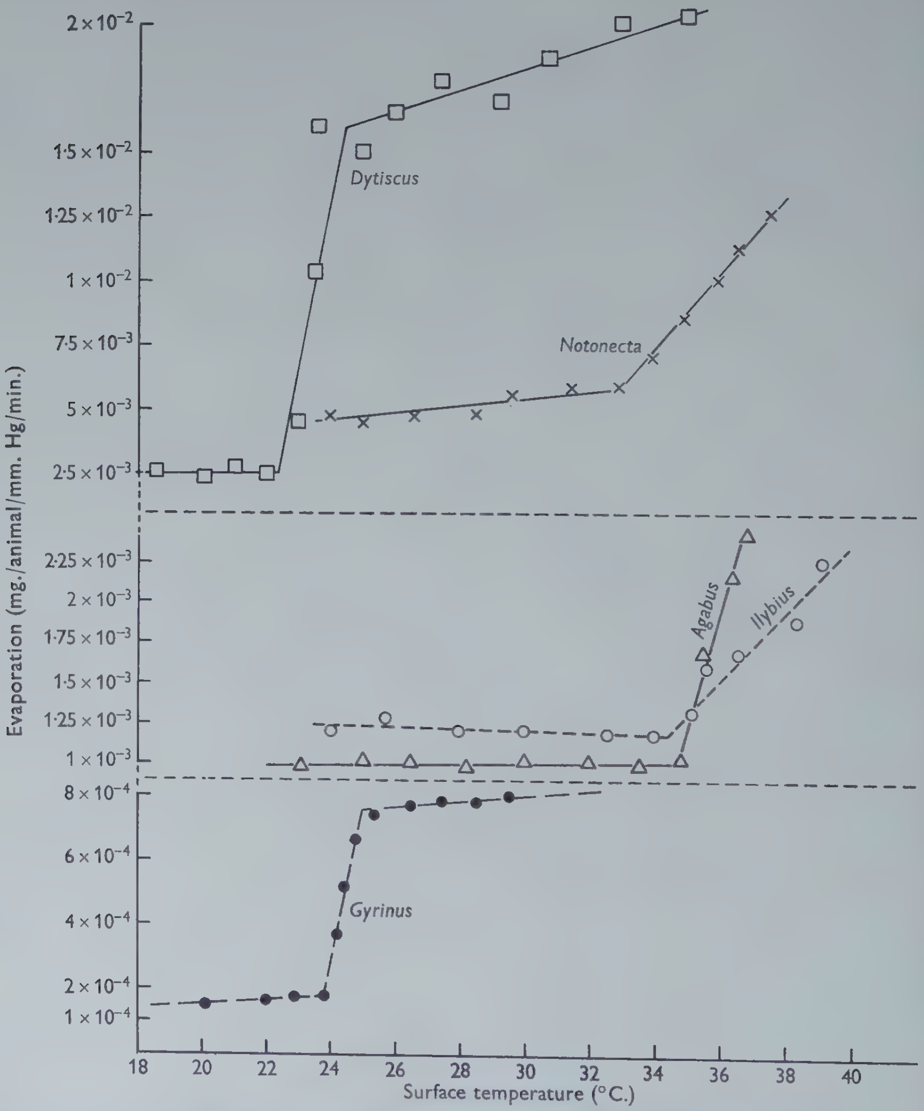

Fig. 1. The relationship between permeability of the cuticle (expressed as evaporation rate per unit of saturation deficiency of dry air) and cuticle surface temperature, for a number of less permeable aquatic insects. *Note.* The ordinate consists of three sections, each with a different linear scale.

and shrunken gill falls rapidly should occasion no great surprise, for the collapse of the structure may limit the supply of water from the haemolymph purely mechanically; there is no need to postulate a change in permeability with desiccation, such as Edney (1951) suggests may occur in isopods. The damselfly (Fig. 3*a*), on the other hand, has two kinds of cuticle with very different temperature coefficients of permeability. Neither the curve for the gill nor that for the thorax shows any indication of transition phenomena. In view of the comparatively high permeability of both cuticles, none would be expected. At lower temperatures the permeability to water of the gill is the greater; its permeability is also relatively unaffected by temperature. As permeability to water is likely to reflect permeability to oxygen also, this may indicate that the gill has the greater respiratory role at lower temperatures. The permeability of the remaining cuticle rises uniformly with temperature, so that at 24° C., the gill is actually less permeable, and by 30° C., the main cuticle may transmit water twice as rapidly as the gill. Both the temperatures quoted are probably above the survival range of this species, but the experiment shows how different the temperature relationships of two parts of the cuticle of one individual may be—and, perhaps, how different their relative roles in, for example, respiration may be at different temperatures.

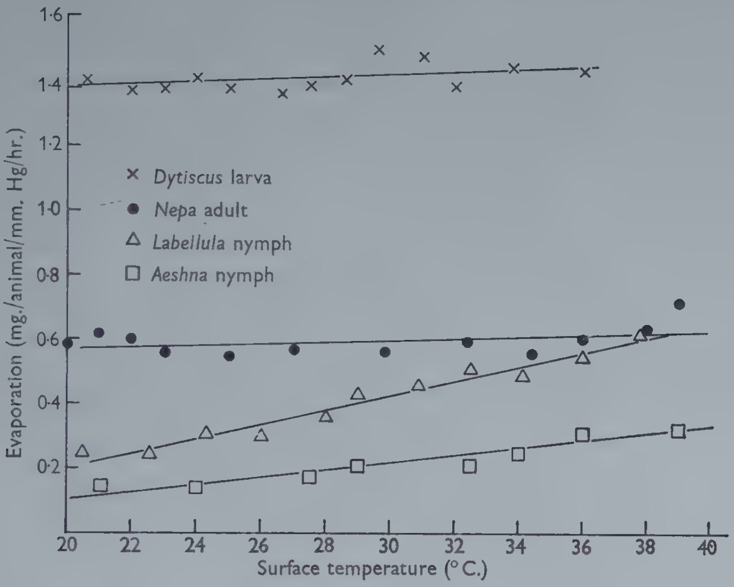

Fig. 2. The relationship between permeability and temperature for a number of more permeable aquatic insects.

The experiments with *Sialis* (Fig. 3*b*) reveal an entirely different state of affairs. The curve for total evaporation against temperature is of complex form, and it would be unwise to infer that either of the irregular portions of it were evidence for a transition phenomenon; they could fall within the limits of experimental error of the method. When the depression of the temperature of the gill is considered, the permeability of this region is seen to be very dependent on temperature. But the permeability of the thoracic cuticle, judged by the change in its temperature depression with rising temperature, is little affected by temperature except for one pronounced discontinuity of Z type, occurring at around 35° C. Interestingly enough, that discontinuity does correspond with one of the small irregular regions of the curve for total water-loss. The validity of the procedure of dissecting the curve for total

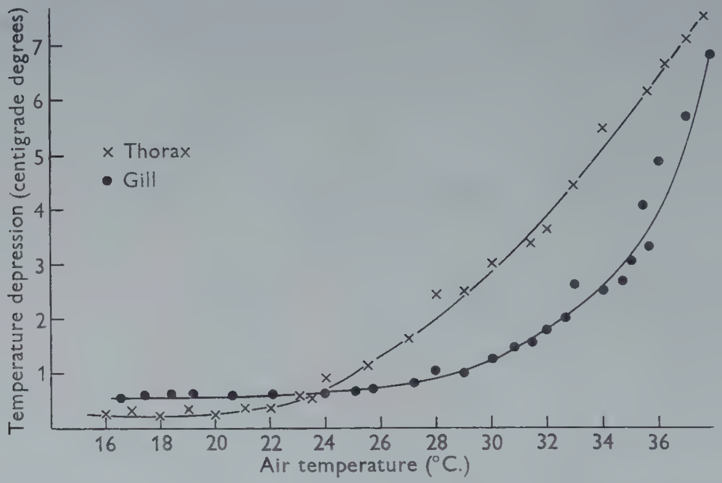

Fig. 3*a*. The relationship between air temperature and the temperature depression (below air temperature) of the cuticle surface, due to evaporation. The curves have been obtained from the thorax and from the gill of *Coenagrion* (Zygoptera, Odonata) larvae.

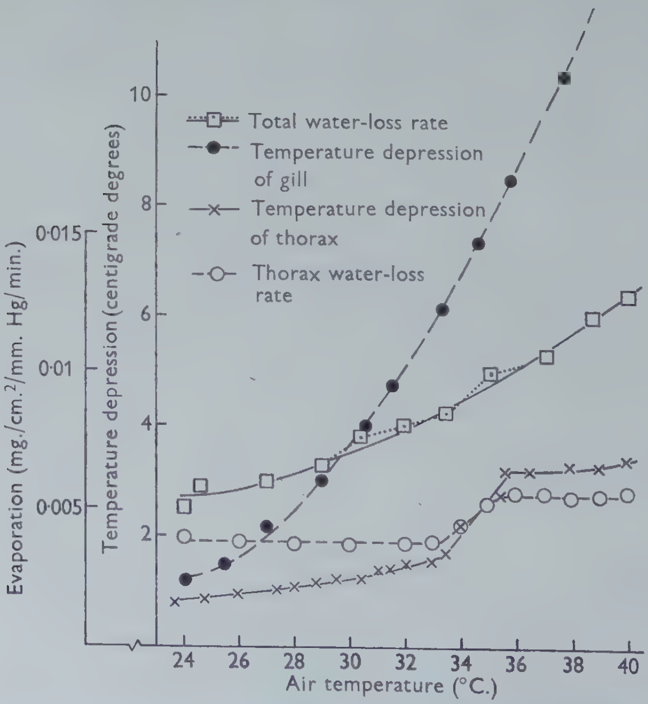

Fig. 3*b*. The relationship between air temperature, surface temperature depression and permeability, for the thorax and for the gill of *Sialis* larvae. For further explanation, see text.

water-loss into its component parts in this manner is particularly supported by the form of the permeability/temperature relationship of the thoracic cuticle, calculated by dividing the values for total water-loss in the ratio of corresponding thoracic to gill temperature depressions as outlined above (p. 282). Though all three functions from which this relationship is derived (total water-loss/temperature, temperature depression of gill/temperature, and temperature depression of thorax/temperature) are of complex form, the calculated curve shows sections which are linear and parallel with the temperature axis, both below and above the transition region; in other words, two simple functions joined by a transition region, with precisely the same form as one obtains from cockroach grease (Beament, 1958*a*) and from the cuticles of *Dytiscus* and *Gyrinus* adults in the present investigation. Furthermore, when specimens of *Sialis* were examined after the experiment, the head and thorax were shiny, as though grease-covered, whereas the abdomen was matt in appearance. There seems little doubt that the gill of *Sialis* (and possibly the whole abdomen) is without an efficient covering of lipid, but the head and thorax have organized grease over them; one could deduce therefore that in this insect the gill is the only region of important respiratory consequence.

DISCUSSION

Even within this small sample of aquatic insect species a wide variety of permeability of the cuticle and of distribution and type of cuticular lipid is present. The animals sharing one specific environment—a pond—may be, like most terrestrial insects, covered by harder wax, or by softer grease, and may be comparatively impervious to water; they may have an effective barrier to water over only a part of their surface, or they may, apparently, be quite devoid of an impermeable lipid barrier. But where organized cuticular lipid is present, all these animals share the property of having a low transition temperature, as compared with many terrestrial forms. It has been argued from the time of the early studies in this field (e.g. Wigglesworth, 1945) that the higher the transition temperature the more impermeable the insect—a correlation confirmed by later investigations (e.g. Lees, 1947; Beament, 1959) and with which present results agree; why impermeability should be so related to the mean molecular weight of the lipid is at present without adequate physico-chemical explanation (see Beament, 1961*a*). To a submerged insect, as compared with an insect exposed to dry air, impermeability may have an entirely different physiological significance, but it is clear that the ecological consequences of very low transition temperatures may be considerable (see Beament, 1961*b*). The adult *Dytiscus* has a transition temperature at around 24° C. It dies in water two to three degrees hotter than this. Dissection suggests that the internal organs are then waterlogged and that death might be attributed to excessive entry of water when the animal becomes less impervious at this higher temperature (a matter which is now under active investigation). Now by comparison, adults of the related beetles *Agabus* and *Ilybius* survive in the laboratory in water at temperatures up to 29° C.; they have very much higher transition temperatures. Whether this observation is reflected in the distribution of these animals in Britain is unknown, but O'Farrell (private communication) says that in Australia *Agabus* is certainly found in water in areas very much hotter than those where *Dytiscus* occurs. Without careful assessment of the characteristics of the actual species (and even varieties) concerned, ideas of this kind must be limited to generalization, but the implication is clear.

From the study of the larvae bearing gills it is apparent that great care must be taken in interpreting the form of a curve representing the total evaporation rate against temperature; indeed, from such a curve alone it may be impossible to deduce anything of consequence. Whatever the property of the rest of the cuticle on such a larva, the gill is very permeable to water—a natural correlate of its primary function of transmitting oxygen efficiently. These nymphs (or perhaps pre-adult stages might be more correct) endure a degree of terrestrial exposure in the process of metamorphosis and adult emergence. In none of the species examined did there appear to be any differences in waterproofing between the final larval stage and the earlier instars; larvae actually in the process of emerging were not examined. There would appear to be a considerable difference between the resistance to desiccation, for example, of the two kinds of dragonfly nymphs; but the atmosphere into which they emerge, immediately over their aquatic environment, must in any case have a very high humidity.

Caution is necessary in interpreting any of the information in this paper in terms of the osmotic protection which waterproofing may confer on the aquatic insect. There can be no doubt that a lipid barrier which restricts evaporation must also reduce the inward flow of water in the submerged insect—but because of the phenomenon of the asymmetric permeability of insect cuticle (Beament, 1961 *a*) we cannot use the values for permeability to outwardly moving water for quantitative estimates of influx rates. In this respect, the most surprising finding in this study is the great difference between the adult beetle and its larva. Both occupy essentially the same habitat and have very similar diets. The larva of *Dytiscus*, for example, has a pair of terminal spiracles, surrounded by hydrofuge hairs, and no obvious adaptations for cutaneous respiration. Yet on present evidence its cuticle is thoroughly permeable to water, and presumably cutaneous respiration could occur. And it must have to offset a considerable osmotic influx of water. The adult is efficiently waterproofed and we have already seen that it cannot sustain the osmotic invasion of its body when that waterproofing loses it efficiency.

Finally, we must consider the actual form of some of the permeability temperature curves which have been obtained, for these suggest reasons for what may otherwise appear as a sporadic representation of lipids. The actual permeabilities of *Dytiscus* and *Gyrinus* adults, below transition, could be the result of a relatively thick layer of molecularly unorganized lipids. The existence of a sharp transition phenomenon eliminates this possibility, whereupon the permeability is consonant with one monomolecular layer alone. Now the increment in permeability at transition (about a fivefold increase) corresponds closely with the characteristics of a monolayer of cockroach grease spread on water, instead of with the behaviour of the much thicker layer of grease on intact *Periplaneta* (Beament, 1958). The permeabilities of *Corixa*, *Notonecta*, *Agabus* and *Ilybius*, all of which show sharply defined transition phenomena, are also of the right order if their surfaces are covered by one monolayer of lipid, though in some of them the form of the permeability/temperature function indicates that the lipid must be a wax rather than a grease. The particular property shared by all the animals mentioned in this paragraph is that they have at least some areas of surface which are highly hydrofuge, even after prolonged immersion in water, and functioning variously as plastons, air-reservoirs, or flotation devices. It may well prove that osmotic protection is not the key to the distribution of cuticular lipids in the aquatic insects, and that highly organized monolayers are associated with their hydrofuge properties. This suggestion will be explored in a later paper in this series.

SUMMARY

1. The permeability to water of the cuticles of a number of aquatic insects has been measured under conditions of evaporation. The range of rates of evaporation amongst these animals is very great; some adult aquatic beetles are quite as waterproof as more permeable terrestrial insects (caterpillars for example), whereas their larvae would seem to be without specific mechanism for restricting the passage of water.

2. Two methods are described, whereby local differences in permeability may be detected, and from one of these methods it is possible to calculate the permeability/temperature characteristics of different areas of cuticle of an intact insect.

3. The more impermeable insects have uniform permeability over their entire surfaces; these include adult beetles, and both mature and immature stages of Hemiptera. Larvae of mayflies and of some beetles, which are very permeable to water, likewise show no local differences in permeability.

4. The gills of all the species examined are very permeable to water; those of zygopteran dragonflies are more permeable than the rest of the cuticle at lower temperatures, those of *Sialis* are much more permeable at all temperatures.

5. The effect of temperature upon permeability has been measured. The more waterproof insects reveal transition phenomena characteristic of organized monolayers of lipid, such as are found in terrestrial insects, but by comparison with them the temperatures at which transition occurs are very low. In particular, the temperature at which adults of *Dytiscus* and *Gyrinus* become suddenly more permeable (*ca.* 25° C.) could obtain in natural circumstances, and the evidence suggests that these animals die through osmotic invasion of water when placed at higher temperatures. Other beetles and aquatic Hemiptera, with higher transition temperatures, survive temperatures above 25° C. These ecological implications are discussed.

6. A transition phenomenon occurs in organized lipid over the main cuticle of *Sialis*, whereas the more permeable gill shows no evidence of this. There is no evidence for organized lipid on the main cuticle of zygopteran larvae, but the temperature/permeability relations of the general cuticle and of the gill are very different.

7. The absolute permeability, and the shape of the permeability/temperature function, of the cuticle of those insects which have very hydrofuge areas of surface suggest that their waterproofing system consists of only one monolayer of organized lipid, which may be either grease or wax.

I am grateful to Prof. A. F. O'Farrell for permission to quote an unpublished observation, and to Prof. V. B. Wigglesworth, F.R.S., and Dr J. Smart for useful discussions. I am indebted to the technical staff of the Subdepartment of Entomology, University of Cambridge, for their painstaking assistance with the collection of material.

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THE INFLUENCE OF WATER-LOADING AND LOW TEMPERATURE ON CERTAIN FUNCTIONAL ASPECTS OF THE CRAYFISH ANTENNAL GLAND*

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INTRODUCTION

The histological structures, which enabled morphologists to deduce the function of the vertebrate glomerular kidney before much of the physiological evidence was available (Smith, 1951), appear not to exist in the crayfish antennal gland. Despite this shortcoming, there exists physiological evidence which indicates that some form of 'filtration'‡ is involved in primary urine formation in that organ. Peters (1935) was able to show that the antennal gland of the crayfish (*Astacus astacus* L.) was responsible for the markedly hypotonic urine of that animal; the chloride content of urine samples removed from various portions of the kidney decreased from proximal (coelomosac, urine *c.* blood-isotonic) to distal (bladder, urine hypotonic). Peters's work did not provide unequivocal evidence that 'filtration' was responsible for primary urine formation, but it did show that that process was a strong possibility. Maluf (1941) studied the clearance of inulin, xylose and various dyes by the kidney of the crayfish *Procambarus clarki* (Girard). He concluded that his data showed the crayfish kidney to be a secretory kidney, since inulin was excreted by that organ in a manner which appeared to be dependent upon a transport mechanism rather than passive filtration. Further, Maluf decided that the crayfish antennal gland showed no threshold response to xylose (a substance which is only a stereotype for glucose according to Martin, 1957). Riegel & Kirschner (1960) have re-examined the role of the antennal gland in the excretion of inulin and glucose by the crayfishes *Orconectes virilis* (Hagen), *Procambarus clarki* (Girard) and *Pacifastacus leniusculus* (Dana). They found that glucose behaved like a threshold susbstance and that phlorizin caused a marked glucosuria. Their work did not rule out the possibility that the crayfish antennal gland is a secretory kidney, but it provided evidence supporting 'filtration' as a more likely mechanism of primary urine formation.

In the present paper are presented the results of studies which were designed to

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‡ The use of the term 'filtration' in this paper follows the restrictions outlined by Riegel & Kirschner (1960). This use of the term implies that urine is formed by the passive movement of fluid from which particles larger than an unknown critical size are restrained, but the cause of the fluid movement is not stated. Where the term is used without quotations, the usual sense of the word is meant—that is, movement of fluid through a porous membrane under the impetus of hydrostatic pressure.

characterize the function of the crayfish antennal gland under conditions of water-loading and low-temperature stress. The experiments were undertaken in order to provide further insight into the physiology of that organ.

MATERIALS AND METHODS

Specimens of *Orconectes virilis* were used in all experiments outlined below. The specimens were maintained as described by Riegel & Kirschner (1960).

Three principal types of experiment were undertaken: (1) urine and sodium excretion of animals into which no injections were made (control experiment); (2) urine and sodium excretion and inulin clearance of crayfishes into which varying amounts of distilled water were injected (water-loading experiments); (3) urine and sodium excretion and inulin clearance of animals under the influence of low temperature (refrigeration experiments).

The procedures for the experiments and the methods of collecting and measuring blood and urine samples, measuring ion concentrations and weight, and making injections were generally the same as those described by Riegel & Kirschner (1960). Differences in procedures and methods between the present and previous work will be noted below as each of the three types of experiment is discussed in detail.

Control experiment

In this series of experiments ten crayfishes were weighed and their urinary bladders (on one side only) were evacuated. At intervals of 4, 8 and 12 hr., all of the urine that had accumulated in the bladder was collected, measured, and samples were saved for sodium analysis.

Water-loading experiments

The bladder on one side was evacuated and injections of either 0·05 ml. (9 animals) or 0·25 ml. (8 animals) of distilled water containing 50 μg. of ^{14}C-inulin were then made. At 4, 8 and 12 hr. intervals after injection all the urine which had accumulated in the bladder was collected. Samples were saved for inulin and sodium analyses. Weight and blood samples were taken before and after the experiments to ascertain that no changes in either blood ions (Na, K and Ca) or weight occurred during the experiments.

Refrigeration experiments

This group of experiments was carried out in two parts. In part one, the bladders of eight crayfishes were evacuated (on one side), the inulin was injected (0·1 ml. of 0·1 % ^{14}C-inulin), and the crayfishes were placed in the water-bath at 16° C. After 4–6 hr. at that temperature the bladders were again evacuated, the total quantity of urine was measured, and samples were saved for sodium and inulin analyses. The crayfishes were then refrigerated gradually (*c.* 2 hr.) to 10° C.; after 6–8 hr. at that temperature, the sample-taking procedure was repeated. The temperature was then lowered gradually to 4° C., and after 8 hr. at that temperature the bladders were evacuated, the total urine output was measured and samples were saved for inulin and sodium analyses. Six of the eight crayfishes were rewarmed to 16° C., the reverse of the foregoing procedure except that warming between 4° and 10° C. and between 10° and 16° C. was not gradual.

In part two of the experiments ten crayfishes were weighed and initial blood samples taken as follows: the ventral membrane between the carpus and merus of the cheliped (claw) was punctured with a fine needle. As soon as sufficient blood had oozed from the wound, a sample was drawn into a 10 μl. pipette and transferred to 2 ml. of deionized distilled water in a 3 ml. test-tube. The micropipette was rinsed in the resulting solution. The crayfishes were then refrigerated gradually (over *c.* 4 hr.) to 4° C. Twenty-four hours later the weighing and blood-collecting procedure was repeated.

By various control procedures, duplicating the actual experiments as closely as possible, it was found that the following average limits of error could be assigned to the various determinations. Blood and urine inulin could be determined within an average error of ±3·6%; blood and urine sodium, calcium and potassium could be determined within average errors of ±0·7%, ±2·0% and ±3·5%, respectively. The weight of the crayfishes could be determined within an average error of ±0·9%. The amount of error involved in the assumption that all the urine produced during a given period of time was collected from the nephropore could not be measured directly. However, that factor was checked as follows: urine was collected from the nephropores of several animals until no more could be obtained. The crayfishes were then sacrificed and the urinary bladders exposed. In all cases, the bladders were collapsed, indicating that all urine but a possible small residual amount had been removed.

RESULTS

Control experiment

The results of the control experiments are shown in Fig. 1. As can be seen, the average urine flow remained relatively constant between the 8 and 12 hr. sampling periods. The excretion of sodium tended to decrease between the two sampling periods.

The urine flow of ten crayfishes averaged 3·0% (S.D. = ±0·75; range = 1·6–4·0) of the body weight per 24 hr.

Water-loading experiments

The results of the water-loading experiments are shown in Fig. 1. The smaller of the two injections of distilled water (0·05 ml.) caused a short-term increase in urine flow, after which the rate of flow fell off to subnormal levels. Concomitant with the fall in urine flow was a decrease in the rate of sodium excretion and in the intensity of water excretion.* The inulin clearance did not change significantly during the 12 hr. period. The large injection of distilled water (0·25 ml.) caused the urine flow to remain relatively constant throughout the experimental period. At the same time the rate of sodium excretion increased enormously and there was a rather large increase in the inulin clearance. The intensity of water excretion decreased during the 12 hr. period, but not to the extent seen in the animals which had been injected with a smaller amount of distilled water.

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* No precedent for the use of the reciprocal of the inulin urine/blood ratio was found in the literature. Since the inulin U/B ratio is commonly used as an indicator of the intensity of water reabsorption, it was felt that the reciprocal of that value could be used to indicate the degree to which water is not reabsorbed, i.e. the intensity of water excretion.

Refrigeration experiments

The results of the refrigeration experiments are shown in Fig. 2. The general effect of low temperature on the crayfishes was seen in decreased urine flow and inulin clearance. There appeared to be a significant difference in the intensity of water excretion between refrigerated and non-refrigerated animals at 10° C., but there was none at 4° C. The rate of sodium excretion during the refrigeration phase of the experiments did not vary significantly from that of non-refrigerated crayfishes. During warming, the rate of sodium excretion of refrigerated animals far surpassed that of animals which had not been refrigerated.

Refrigeration to 4° C. over a 24 hr. period effected no changes either in weight or in blood ions (Na, K and Ca).

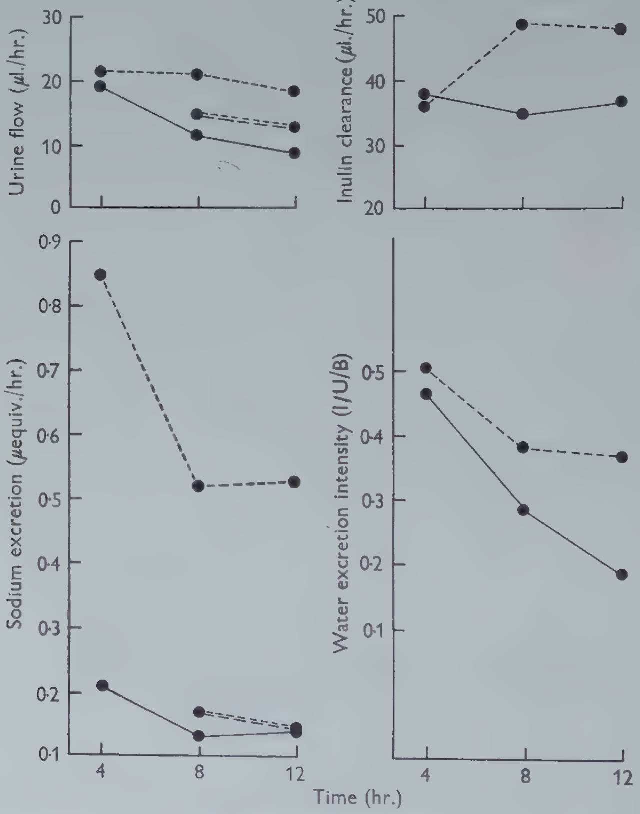

Fig. 1. Average values of urine flow, inulin clearance, rate of sodium excretion and intensity of water excretion in relation to time in crayfishes that had been injected with distilled water (●- - -● = 0·25 ml. injected; ●—● = 0·05 ml. injected). Also shown are average values for urine flow and sodium excretion of uninjected crayfishes (●======● = controls).

DISCUSSION

Normal urine production of Orconectes virilis

As shown in the results, the urine flow per 24 hr. for specimens of *Orconectes virilis* averaged 3 % of the body weight. Whether or not that value represents the normal urine flow is not known. The procedure used for collecting urine put a great deal of stress on the crayfishes. This was shown by the somewhat erratic urine flows in all parts of the present experiments. The erratic results were most pronounced in the control experiments, but they also manifested themselves in the water-loading and refrigeration experiments. One of the stress phenomena exhibited by crayfishes is a cessation or reduction in urine flow [see Riegel (1960) and Riegel & Kirschner (1960)].

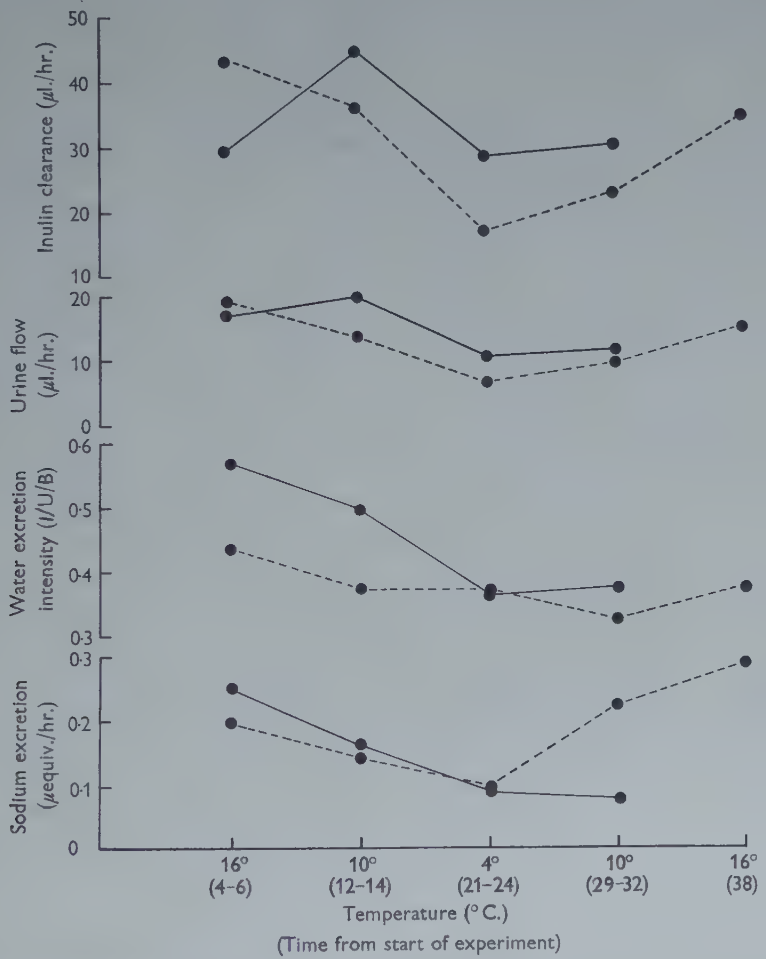

Fig. 2. Average values of urine flow, inulin clearance, rate of sodium excretion and intensity of water excretion of refrigerated and rewarmed crayfishes (●– – –●) and unrefrigerated controls (●——●) in relation to time and/or temperature.

The urine flow of *Procambarus clarki* has been determined by Maluf (1941) and Lienemann (1938) as approximately 5 % of the body weight per 24 hr. These investigators determined the urine flow by plugging the nephropores and either noting the change in weight (Maluf, 1941) or opening the nephropores after a period and aspirating the urine from them (Lienemann, 1938). Numerous determinations of the inulin clearance of specimens of *P. clarki* (unpublished) have been carried out by the writer.

The average inulin clearance amounted to 14·4% of the body weight per 24 hr. The usual inulin U/B ratio in specimens of that species varied between 2·5 and 3·0 (at 16° C.). Therefore, it appears that the urine flow calculated from inulin clearance and inulin U/B ratios agrees with the urine flow determined independently by Maluf and Lienemann. The average inulin clearance found in specimens of *Orconectes virilis* was 6·4% of the body weight per 24 hr. Since the usual inulin U/B ratio of such specimens varied between 2·0 and 2·5, the independently obtained value of 3% of the body weight for urine flow per 24 hr. agrees with the urine flow calculated from average values of inulin clearance and inulin U/B ratios.

Water-loading

From Fig. 1 it may be seen that the injection of distilled water into crayfishes caused a pronounced diuresis, accompanied by an increased rate of sodium excretion. When a small water-load (0·05 ml.) was injected, the effect was short-termed. After the first 4 hr. of the experiment the inulin clearance remained relatively constant while the urine flow, the rate of sodium excretion and the intensity of water excretion decreased markedly. The above observations suggest that some physiological limit may have been exceeded during the first 4 hr. of the experiment. That is, it is possible that the crayfishes overcompensated for the water injected, perhaps by excreting too large a quantity of salt (sodium), in an effort to rid the body of the water. That is certainly suggested by the fact that the levels of sodium excretion were in excess of those in the control group. After 4 hr. the urine flow, the rate of sodium excretion and the intensity of water excretion decreased in the lightly water-loaded crayfishes. Since the inulin clearance remained steady, it is probable that the decreases in the aforementioned parameters were the result of compensatory adjustments under which possible deficits in salts (and water), incurred during the diuresis, were made up. In crayfishes into which the large quantity (0·25 ml.) of distilled water was injected, the results were similar in kind to those obtained with the lightly water-loaded crayfishes, but they differed in degree. The results suggested that the heavily water-loaded crayfishes were unable to excrete the water in the 12 hr. experimental period, despite the marked increase in the inulin clearance, and that in consequence it was necessary for them to excrete quantities of sodium much larger than normal. The overcompensation in urine flow, rate of sodium excretion and intensity of water excretion was not seen in the heavily water-loaded crayfishes, although those parameters did decrease after the first 4 hr. of the experiments.

In summary, crayfishes are able to excrete relatively large quantities of distilled water injected into them. However, in the process, it seems likely that they must suffer the loss of abnormal quantities of salt (sodium), which must be compensated for, after the body is rid of the excess water, by greatly increased reabsorption of salts (sodium) from the urine.

Burger (1957), in his study of excretion in the lobster, *Homarus americanus*, encountered animals which normally did not produce urine. He found that those anuric lobsters could be induced to produce erratic, transitory urine flows by injecting hyper- or hypotonic salt solutions into them. That fact seems puzzling when one considers that urine formation in the lobster could not be correlated with a rise in the arterial pressure (Burger, 1957). In view of this observation on the lobster caution must be exercised in making any statements about the causes of the increased urine flow in the crayfishes studied here. It is possible that the extra water, irrespective of the accompanying salt, is able to elicit the diuretic response.

Low temperature

The primary effects of low temperature on the excretory processes of the crayfishes appeared to be the results of a general interference with the metabolism of the experimental animals. Thus the inulin clearance decreased and there was a concomitant decrease in urine flow. The rate of sodium excretion appeared to vary little from that of non-refrigerated crayfishes. During rewarming, however, the rate of sodium excretion of the refrigerated crayfishes increased markedly. From the latter observation it seems possible that there was an accumulation of sodium during refrigeration.

Wikgren (1953) studied the effect of low temperature on the ion balance of the crayfish *Potamobius fluviatilis* (which probably corresponds to the present-day species *Astacus astacus*). Low temperature had the effect of causing a pronounced loss of ions from the crayfishes. Wikgren deduced that the loss was due to the inability of the ion-absorbing surfaces to keep pace with the diffusion of ions out of the crayfishes, rather than a many-fold increase in the concentration of ions in the urine. The results of the present experiments confirm Wikgren's deduction and show, further, that if an ion loss occurred at low temperature, it was not accompanied by a decrease in the ion concentrations of the blood or by change in weight (i.e. water-loss or gain).

As far as can be ascertained, no research into the effect of low temperature on known secretory kidneys has been undertaken. However, a number of investigations have been made of the effect of low temperature on animals in which it is well established that filtration plays a role in urine formation. Potts (1954) studied the influence of low temperature on the clearance of inulin by the lamellibranch mollusc, *Anodonta cygnea* L. Low temperature markedly reduced the inulin clearance. In the lamprey, *Petromyzon fluviatilis* L., low temperature had effects remarkably similar to those seen in the crayfish. According to Wikgren (1953), the urine flow decreased considerably, there was an initial loss of salts from the surface, which reached a steady-state soon after, and the ion (chloride) concentration of the urine did not differ significantly from its value at room temperature. Jørgensen (1950) studied the effect of low temperature on osmoregulation in the frog, *Rana esculenta* L. Low temperature caused a reduction in urine flow and an accumulation of salt (followed by an increase in weight). Jørgensen concluded that the effects were due to the inability of the frog kidney to eliminate salt at low temperatures. If hypotonic (to the blood) saline was injected into the frogs, a diuresis and weight loss would result. However, injection of hypertonic saline caused a complete stoppage of urine flow and a weight increase.

General

The results of the experiments on water-loading and low-temperature effects on the crayfish antennal gland provide data which further characterize the physiology of that organ. Unfortunately, the data do not provide primary evidence as to the mechanism by which that organ forms the primary urine. The results of the water-loading experiments were much as would be expected on the assumption that urine is formed by 'filtration'. If the crayfish antennal gland were a secretory kidney, the injection of large amounts of water would not be expected to have such a pronounced effect on the urine flow and inulin clearance. As seen in the lightly water-loaded animals, the inulin clearance remained constant even when the urine flow dropped below normal. On the assumption that urine is formed by secretion, that observation might be rationalized to indicate that a maximal transport rate for inulin had been reached. However, the inulin clearance seen in the lightly water-loaded crayfishes apparently was not maximal, since that rate increased considerably in the heavily water-loaded crayfishes. Therefore, any attempt to explain the data obtained from the water-loading experiments on the basis of secretion as the mechanism of primary urine formation in crayfishes would seem highly dubious.

The response of the crayfish kidney to low temperature shows many similarities to responses shown by the kidney of a lamellibranch mollusc (Potts, 1954) and the mesonephric (glomerular) kidneys of vertebrates (Jørgensen, 1950; Wikgren, 1953). Nevertheless, however well the present results may agree with those seen in kidneys known to produce urine by filtration they fail to provide decisive evidence as to the method of primary urine formation in the antennal gland of the crayfish.

SUMMARY

1. The influence of water-loading and low-temperature stress on the physiology of the antennal gland was studied in specimens of the freshwater crayfish, *Orconectes virilis*.

2. The results of experiments on water-loading suggested that the crayfishes were able to excrete water injected into them, but they did so at the expense of an abnormal loss of salts (sodium). Heavy water-loads caused large and prolonged increases in inulin clearances and urine flow.

3. Low temperature had its primary effect in reducing urine flow and inulin clearance. Low temperature apparently had little effect on the rate of sodium excretion or intensity of water excretion.

4. The urine flow (at 16° C.) of specimens of *O. virilis* was determined to be 3% of the body weight per 24 hr. This value agreed with the urine flow calculated from average inulin clearances and inulin U/B ratios obtained independently upon specimens of the same species.

5. The results presented in the present paper throw further light upon the function of the crayfish antennal gland. They agree fairly closely with results obtained in other animals where filtration is known to play a role in primary urine formation. However, because of limitations imposed, principally by the lack of morphological evidence for a filtration site in the crayfish kidney, it cannot be stated unequivocally that the crayfish antennal gland is a filtration kidney.

The writer wishes to express his gratitude to Dr Leonard B. Kirschner, of Washington State University, for permitting free use of his facilities during the period of this study. Thanks are also given to Dr A. P. M. Lockwood, of Cambridge University, and Dr P. A. Dehnel, of the University of British Columbia, for reading portions of the manuscript.

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ON THE REGULATION OF THE RESPIRATION IN REPTILES

I. THE EFFECT OF TEMPERATURE AND CO_2 ON THE RESPIRATION OF LIZARDS (*LACERTA*)

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Ventilation of the lungs accomplished by movements of the ribs is, in the phylogenetic development of the animals, first found in the reptilian class. It may therefore be of interest to investigate the regulation of respiration in this group. The reactions to carbon dioxide and to low oxygen tensions is specially interesting considering the importance of these substances for the respiratory regulation in higher animals and man.

The literature on reptilian respiration is not extensive and is mostly concerned with the respiratory movements in different species: Bert (1870), turtles, snakes, lizards, crocodiles; Langendorff (1891), *Lacerta, Anguis*; Siefert (1896), *Lacerta*; Kahn (1902), *Lacerta*; Babak (1914*a*, *b*), *Iguana*, crocodile; v. Saalfeld (1934*a*, *b*), *Uromastix*; Willem & Bertrand (1936), *Lacerta*; Vos (1936), turtles, snakes, lizards, crocodiles; and Boelaert (1941), various lacertilians, and (1942), crocodiles.

The chemical regulation of respiration in various species of reptiles has earlier been studied by Siefert (1896), Babak (1914*a*, *b*), v. Saalfeld (1934*a*), Vos (1936), Boelaert (1941) and Randall, Stullken & Hiestand (1944). A critical survey of the literature was presented by Vos (1936).

The more recent investigations confirm the finding that CO_2 and lack of O_2 have a strong effect on the respiratory pattern of reptiles, but the various studies are often ambiguous and incomplete. Mostly, very high CO_2 and very low O_2 concentrations have been used. The range of concentrations within which a natural regulatory effect of these gases take place is probably much smaller. Investigations of the effect of temperature on respiration are few, and the relation between O_2 uptake and pulmonary ventilation has never been studied.

In the present work, the relationships between temperature, O_2 uptake, and ventilation have been studied; further, the effect of different concentrations of CO_2 in the inspiratory air on respiration has been investigated by a method which gives simultaneous determinations of pulmonary ventilation and oxygen uptake. Two species of lizard (*Lacerta viridis* and *L. sicula*) were used as experimental animals. Their reactions to the respiratory stimuli applied were completely identical.

I. METHODS AND PROCEDURE

The method employed records the changes in volume of the body as a whole, which is the same as a registration of the volume changes in the lungs caused by the pulmonary ventilation. By this method, unanaesthetized animals can be used in repeated experiments. This is an advantage when the natural respiratory regulation is to be studied.

Apparatus. The experimental set-up is shown in Fig. 1. *A* is a spirometer (volume ½ l. or 1·2 l.) loaded with a weight *l*, so that air is forced out through the resistance *x* through the helmet of the animal container *E* and out past the glass syringes *J*. By changing the weight on the spirometer and the resistance (glass capillaries of different lengths and bores) the airflow through the system can be varied. On the drum *B* the air content in the spirometer *A* is registered and the volume of air forced through the system during the experiment can therefore be measured.

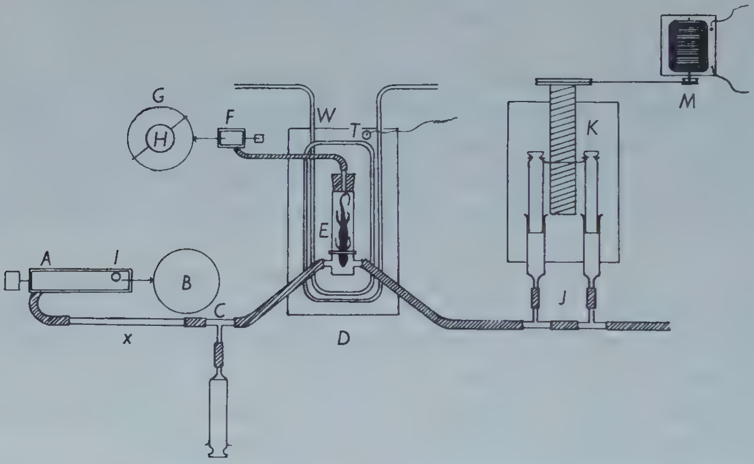

Fig. 1. Apparatus. *A*, spirometer; *B*, drum; *C*, test-tube for air sampling; *D*, water bath; *E*, animal container; *F*, small Krogh-type spirometer for registration of the respirations; *G*, revolving drum; *H*, clockwork; *J*, air-sampling syringes; *K*, sampling device; *M*, electric motor; *T*, contact thermometer of the thermostat; *W*, cooling spiral. *l*, weight; *x*, glass capillary resistance.

C is a test-tube for taking samples of the influx air. *D* is a container filled with water. The temperature within the container can be regulated by leading cold water through the coil *W* or by electrical heating. In the latter case, the thermostat *T* keeps the temperature constant ($\pm\frac{1}{10}$° C.). A small electric motor drives a stirrer and provides an even temperature distribution in the water.

E is the animal container and *F* is a small Krogh-type spirometer (volume 3·5 ml. or 7 ml.) registering the respiratory movements on the drum *G*. *G* is turned at a constant speed (one turn per 30 min., or one per 15 min.) by a clockwork motor *H*.

The electric motor *M* drives the sampling device *K* which slowly pulls back the pistons in the syringes *J*, so that a small part of the air coming from the animal container (the exit air) is continually sampled.

The animal to be used was placed in a separate cage without food and water the day before the experiment. During the experiment, the animal was placed with a tight-fitting rubber diaphragm around its neck, in a container consisting of a body-chamber and a 'helmet'. The helmet and body-chamber, which were firmly screwed together, were thus separated from one another by the air-tight diaphragm.

The animal breathed the air flowing through the helmet, and the volume changes of the body in the body-chamber were transmitted to the spirometer *F* and registered as a ventilation curve on the revolving drum *G*. The animal container was placed in the water bath *D*, so that the experiment could be performed at constant temperature.

At each experiment the spirometer *A* was filled with the desired air mixture. The flow was so regulated that the difference in CO_2 percentage in the air reaching the helmet (influx air) and leaving the helmet (exit air) was about 0·6 %.

When air mixtures different from room air were used, the particular air mixture was bubbled through the water in the spirometer for about 30 min., so that equilibrium between water and air in the spirometer could be established before the experiment. Each experiment lasted 15 min., but readings and sampling were not started until an initial period (15–60 min.) had elapsed, during which the body temperature became constant and the respiration regular.

Immediately before and after the actual experiment, samples of the influx air were taken. The body temperature of the animal could be measured by means of a thermocouple, one junction of which, mounted in a small plastic tube, was inserted 1 cm. into the cloaca of the animal. Usually two experiments were made in succession without touching the animal, which often spent about 2 hr. in the apparatus.

Double analyses for CO_2 and O_2 content of the influx and exit air were made on the Scholander 0·5 ml. analysing apparatus (Scholander, 1947). If the results of a pair differed more than 0·05 % on CO_2 or O_2 the experiment was discarded.

Computations. From the volume of the influx air at STPD and the N_2 percentages of the influx and exit air, the volume of the exit air was calculated. Oxygen uptake, carbon dioxide elimination, and R.Q. could then be determined. On the respiration curve, the number of respirations during the experiment was counted and the depth of the respirations was measured. From these data, an average respiratory frequency and an average respiratory depth were estimated. The product of these gave the average pulmonary ventilation per minute during the experiment. (The values were converted to BTPS.)

Accuracy of the method. The accuracy of the results attained by the described method depends on the accuracy of the readings of the volumes of air from spirometer *A* and on the reliability of the analyses. The latter is of special importance when, as here, the difference between influx air and exit air used for computing the metabolism is so small (0·6 %).

The error on the volume readings is only about 1 %, and the uncertainty of the values for the metabolism can be calculated to be about 10 %. The error on the ventilation is of the same order of magnitude, roughly estimated to be 10 %, and depends mostly on the readings of the respiratory amplitude.

Even if the values of both metabolism and respiration obtained by this method are encumbered with the above-mentioned uncertainties, they give valuable information. The advantage of giving absolute values for respiratory frequency, depth, and ventilation under different circumstances must be considered great compared to the mere description given in earlier investigations.

II. VENTILATION AND METABOLISM AT DIFFERENT TEMPERATURES

In ten different animals (3 *Lacerta sicula* and 7 *L. viridis*) the oxygen uptake and the pulmonary ventilation were determined as described in Part I.

The experimental conditions were, in all experiments, kept as near to resting conditions as possible. It was very seldom that the animals did not struggle to get free one or more times during the experiment. It must also be mentioned that an animal held (as in the present experiments) in a fixed position is probably not relaxed and has an increased muscle tone which may increase the oxygen uptake above the basal level. However, as these sources of error are present in all the experiments, it is still justifiable to compare them.

The oxygen uptake was varied by changing the body temperature of the animals. It usually took 40–60 min. to cool an animal down from room temperature to 10° C., and about the same time to warm it up to 35° C. Experiments were performed at temperatures of 10°, 20° (room temperature), 30° and 35° C. The relationship between O_2 uptake and temperature is shown in Fig. 2.

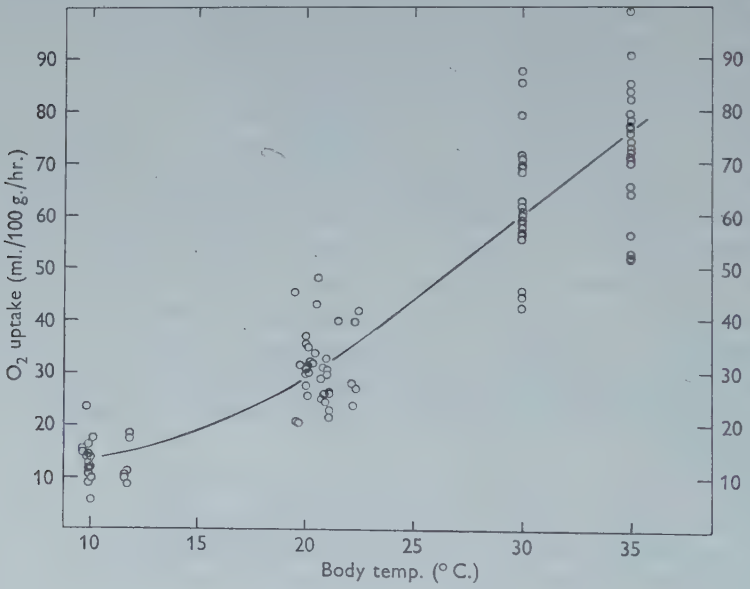

Fig. 2. Oxygen uptake in ml. per 100 g. per hour in relation to body temperature °C. (results from eight animals).

RESULTS

In Fig. 3 the pulmonary ventilation (BTPS) is plotted in relation to the oxygen uptake (STPD). An increase in oxygen uptake gives an increased pulmonary ventilation. The relation is not quite rectilinear.

The oxygen uptake and the ventilation at 10° C. are relatively low. This is probably caused by the lack of struggling at this temperature where the animals are sluggish. In Fig. 4 the respiratory frequency and the amplitude at different temperatures are shown. The respiratory frequency increases with increasing body temperature. The increase seems to be exponential. The depth of the respirations is independent of changes in temperature. In another series, performed on another set of animals, the respiratory depth varied more (as seen in Fig. 5, where the results are given as percentages of the 35° C. value). No relationship, however, seems to exist between

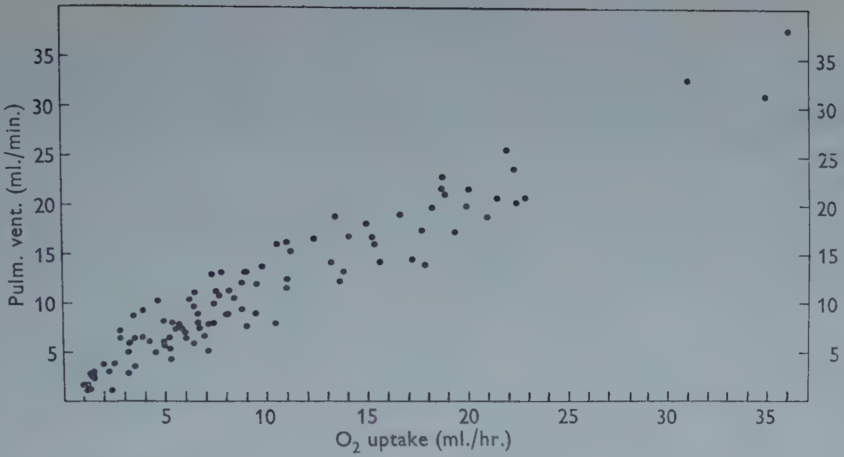

Fig. 3. The relationship between pulmonary ventilation, ml./min. (BTPS) and oxygen uptake, ml./hr. (STPD). The oxygen uptake was varied by changing the body temperature of the animals (results from eight animals combined).

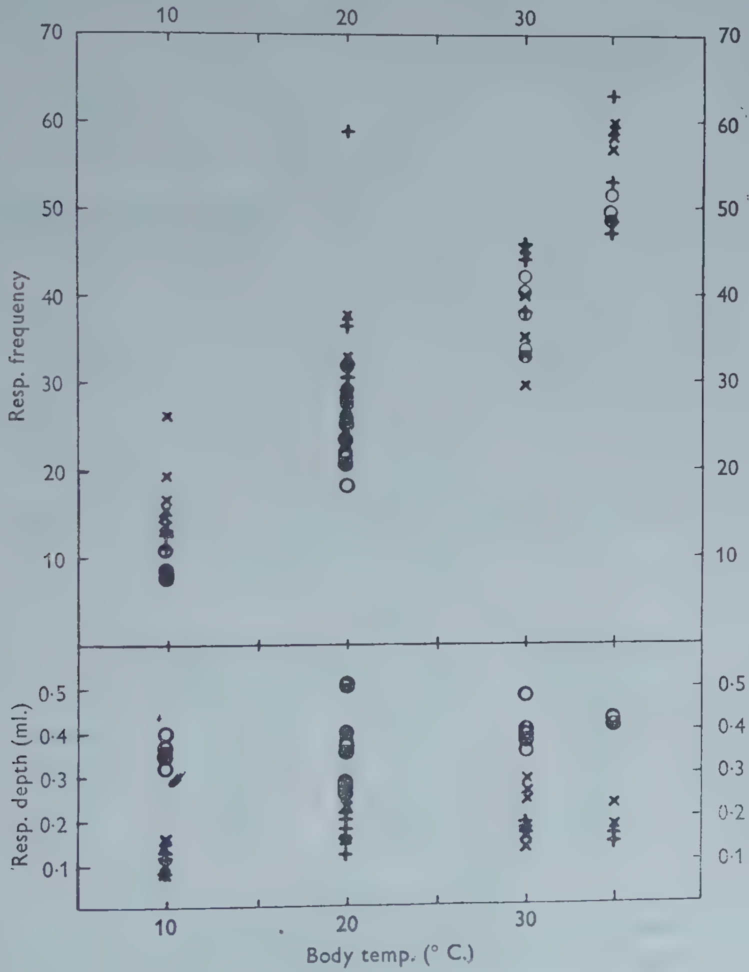

Fig. 4. Above: respiratory frequency in relation to body temperature. Below: respiratory depth in relation to body temperature (four animals: ○, ●, + and ×).

temperature and respiratory depth. The quotient, *ventilation/hour* ÷ O$_2$ *uptake/hour* (i.e. the ventilation per litre consumed oxygen), varies a great deal even for the same animal in repeated experiments. Mean values for all animals are shown in Table 1, column 3.

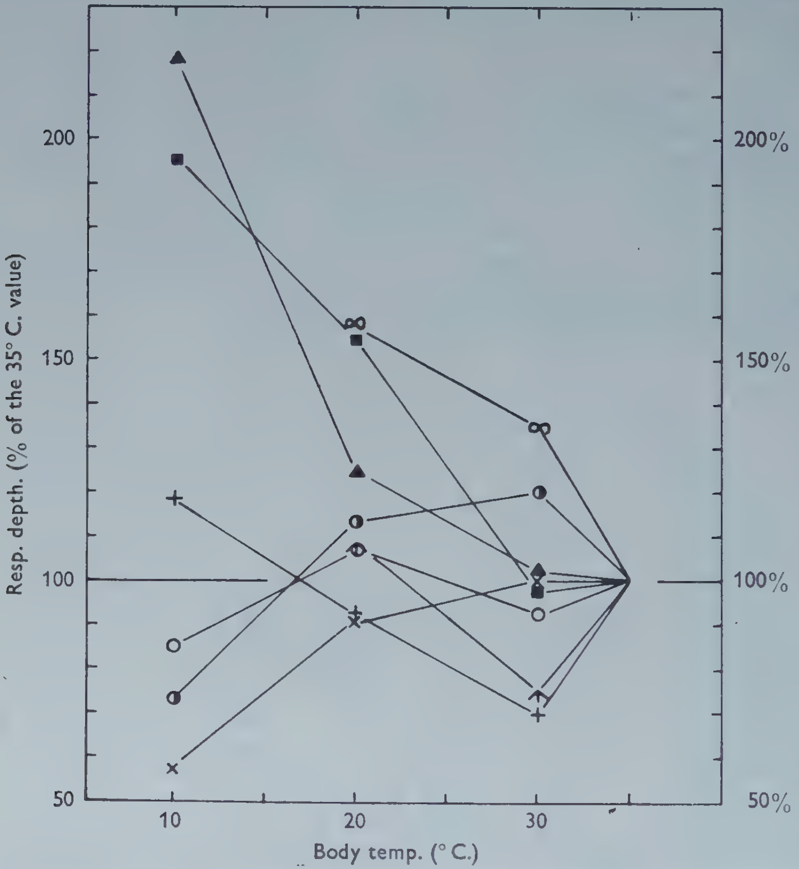

Fig. 5. Respiratory depth in percentage of the 35° C. value in relation to body temperature (eight animals: ○, ×, ◑, +, ▲, ■, ∞ and ↑).

Table 1

Temp. (°C.)	No. of experiments n	Vent./hr. (BTPS) / O$_2$ uptake/hr. (STPD), mean m	Standard error of the mean s	Vent./hr. (STPD) / O$_2$ uptake/hr. (STPD), mean m_1	R.Q. mean	Vent./hr. (ST / CO$_2$ output/ (STPD), mean
ca. 10	22	96·8	±7·89	92·1	0·88	104·7
ca. 20	38	87·2	±3·81	79·2	0·80	99·0
30	23	65·4	±2·93	56·0	0·81	69·1
35	23	66·0	±2·05	55·2	0·82	67·3

The differences between the ventilation quotients at 10° and 30° C., 10° and 35° C., 20° and 30° C., and at 20° and 35° C. are highly significant, while the differences between the quotients at 10° and 20° C., and 30° and 35° C. are not statistically significant.

The high numerical value of the quotient (65–97, as compared to 20–24 in man) is probably due to the rather primitive structure of the lungs (cf. Milani, 1894).

DISCUSSION

This part of the investigation showed that the rise in metabolic rate caused by an increase in temperature is accompanied by an increase in pulmonary ventilation. The increase is brought about by an increase in respiratory frequency, whereas the respiratory depth remains practically unchanged.

The increased ventilation may be caused by an increased blood pCO_2 or by the increased temperature *per se*, or, perhaps, by a combination of both factors. An increased blood pCO_2 could be caused simply by the increased production of CO_2 at the higher temperatures. No values of blood pCO_2 are available, but certain conclusions as to its variations may be drawn from the experimental results.

The CO_2 output per minute can be expressed as:

$$CO_2 \text{ production} = \text{pulmonary ventilation (STPD)} \times (\text{expired } CO_2\,\% - \text{inspired } CO_2\,\%),$$

from which follows that

$$\frac{\text{pulm. vent. (STPD)}}{CO_2 \text{ production (STPD)}} = \frac{1}{(\text{exp. } CO_2\,\% - \text{insp. } CO_2\,\%)}.$$

The left-hand expression decreases as temperature increases (Table 1, column 7). Consequently, (exp. $CO_2\,\%$ – insp. $CO_2\,\%$) must be increasing. As the inspired CO_2 percentage is kept nearly constant (it must be equal to the average CO_2 percentage in the helmet, which again is approximately equal to that in the exit air), it follows that the CO_2 percentage in the expired air must be higher at the higher temperatures. With a constant respiratory depth (and a presumably constant dead space) it can be concluded that the alveolar CO_2 percentage and hence the blood and tissue pCO_2 is also higher at higher temperatures. The increased ventilation must, therefore, at least partly be caused by an increased CO_2 stimulus on the respiratory centre.

This effect of the increased tissue and blood pCO_2 on respiration is apparently different from the effect of CO_2 added to the inspired air in that it increases the respiratory frequency and ventilation, whereas inspired CO_2 increases the respiratory depth and slows the frequency as will be discussed in Part III.

An effect of increased temperature alone on respiration has been demonstrated by v. Saalfeld (1934*a*). He found that local heating of a leg of *Uromastix*, from which the skin was removed and all nerve connexions cut, was followed by an increased ventilation. This effect could be prevented if the neck of the animal (carotid and vertebral arteries) was cooled. From this he concluded that the rise in pulmonary ventilation was caused by a heating of the respiratory centre by the blood. He assumed that, by a general heating of the animal, metabolites from the increased metabolism should further act as stimuli for the respiratory centre, as cooling in this case did not completely abolish the ventilatory increase.

In humans, Cunningham & O'Riordan (1957) have found that a raised temperature increases the sensitivity of the respiratory centre towards CO_2. An interaction of this kind between temperature and CO_2 might, in the case of *Lacerta* also, be one of the reasons for the increase in pulmonary ventilation at higher temperatures. Finally, metabolic factors other than CO_2 could be involved in the ventilatory response to increased temperature. Whether the increase is caused by one or several of the above-mentioned factors cannot be decided at present. Determinations of pCO_2 in blood and alveolar air may give interesting results.

In the temperature interval studied here the rise in pulmonary ventilation is not connected with temperature regulation. The rise is called forth by the metabolic requirements. This can be seen from the ratio *pulmonary ventilation/litre of* O_2 *consumed.* This quotient is not higher at higher temperatures as it would have been if the ventilatory rise was due to thermal panting. On the contrary, the value of *ventilation (BTPS)/hr.* $\div$ O_2 *uptake (STPD)/hr.* at both 30° and 35° C. is smaller than at 10° and 20° C. At still higher temperatures it is quite possible that *Lacerta* also would show thermal panting [cf. Langlois (1901), Cowles & Bogert (1944), and others].

The better utilization of the respiratory air found at higher temperatures (cf. ventilation (STPD)/O_2 uptake (STPD), Table 1) may be due to a larger, and perhaps better distributed, blood flow through the lungs. It cannot, however, be due to a relatively decreased anatomical dead space, as the respiratory depth is not greater but remains unchanged or even becomes smaller at 30° and 35° C.

III. PULMONARY VENTILATION AND OXYGEN UPTAKE AT DIFFERENT CO_2 PERCENTAGES

Five lizards (2 *Lacerta sicula* and 3 *L. viridis*) were used for the study of the influence of CO_2 on respiration. The procedure is described in Part I. Pulmonary ventilation, respiratory frequency and depth, and oxygen uptake were measured.

The composition of the actually inspired or expired air is not known and cannot be computed from the values measured in the experiments. The exit air, however, must fairly accurately represent the average composition of the air in the helmet from which the animal inspires. By changing the flow rate and the composition of the influx air, the exit air can be maintained at a relatively constant composition for any CO_2 percentage desired.

The CO_2 percentage of the expired air and the alveolar air is naturally higher than that of the exit (and influx) air. At a constant CO_2 production (i.e. rest at constant temperature) an increase in CO_2 percentage in the helmet (exit air) would produce an equal increase in the expired air if the ventilation did not change. If, however, the ventilation increases, the increase in CO_2 percentage of the expired air will be less than that of the exit air.

In the following paragraphs the changes in pulmonary ventilation and respiratory pattern will be related to the CO_2 percentage in the exit air. It must be understood that the changes in alveolar CO_2 percentages may be smaller than those of the exit air, i.e. in cases where the alveolar ventilation has increased.

RESULTS

When the CO_2 percentage of the exit air is increased, the respiratory pattern changes. CO_2 percentages below 3% in the exit air causes a gradual increase in respiratory depth and pulmonary ventilation and a decrease in respiratory frequency.

When the CO_2 percentage is increased above 3–4 % it produces a 'periodic inhibition' of respiration. Groups of respirations separated by inspiratory pauses lasting up to 1 min. occur. The duration of this periodic inhibition ('Cheyne-Stokes-like' respiration) is dependent on the CO_2 percentage, lasting from a few minutes at 3 % to more than 1 hr. at 13·6 %. In this state the respiratory depth is increasing, while the pulmonary ventilation naturally is very low due to the disturbed breathing rate. This is illustrated by Figs. 6*a*, 7 and 8, where the effect of 7·2 % CO_2 is shown.

After 20–60 min. respiration always becomes adjusted to the CO_2 percentage and is unchanged and regular from then on (steady state, Fig. 6*b*).

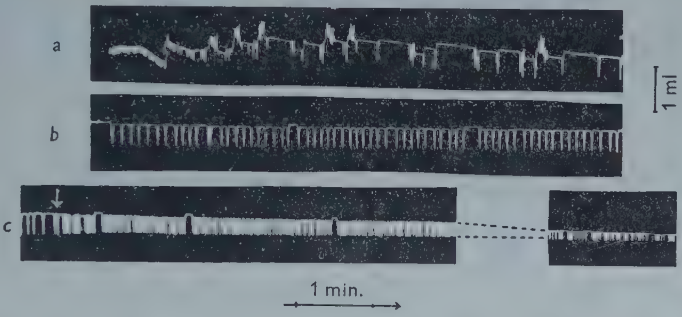

Fig. 6. Respiration curves: (*a*) Start of CO_2 breathing, note Cheyne–Stokes respiration and long inspiratory pauses. (*b*) Steady state, 7·2 % CO_2 in exit air. (*c*) Change from CO_2 breathing to room air breathing at ↓ (read left to right).

After a steady state has been reached, a sudden shift back to room air with quick flow causes an instantaneous increase in respiratory frequency and, consequently, also in pulmonary ventilation (cf. Figs. 7 and 8). The ventilations attained here are the highest registered for the animals concerned. The respiratory frequency then decreases gradually, reaching the normal steady-state value after 2–10 min.

During the same time the respiratory depth after the shift to room air decreases slowly and, consequently, the pulmonary ventilation also decreases. Normal values for respiratory depth and ventilation are reached after 20–30 min. (Figs. 7, 8).

In Fig. 9 the pulmonary ventilation in the steady state is plotted in relation to the CO_2 percentage in the exit air. It is seen that an increase in CO_2 to about 3 % causes a slight increase in pulmonary ventilation. At further increases in the CO_2 percentage the ventilation again decreases, even to subnormal values. This maximum, at about 2·75 % CO_2, was observed in all five animals investigated. In the most CO_2-sensitive animal the ventilation was doubled at this CO_2 concentration.

Fig. 9 shows that in the steady state the respiratory depth increases regularly with increasing CO_2 percentage in the exit air (0·4–13·6 %), while the respiratory frequency decreases. These relationships between CO_2 and respiratory depth and frequency was found to be unaffected by a change in the animal's body temperature from 20° to 30° C. The shape of the curves obtained at 30° C. was similar to that of the 20° C. curves, the frequency curve lying higher, and the respiratory depth curve a little below the corresponding 20° C. curves.

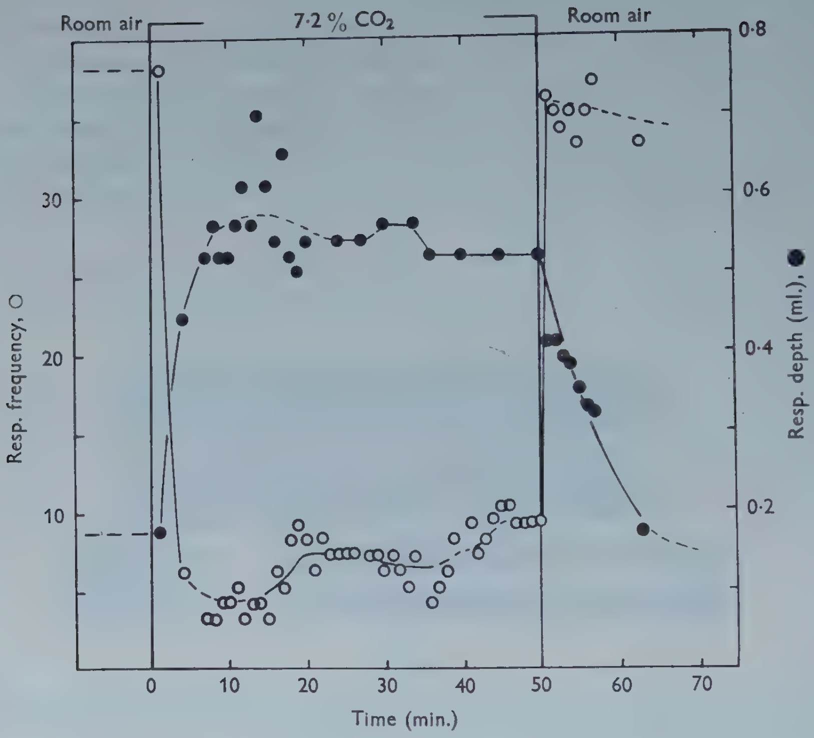

Fig. 7. Respiratory changes produced by changing from room air breathing to CO_2 breathing (50 min.) and back to room air. CO_2 percentage 7·2 in exit air. Time in minutes. ○, Respiratory frequency; ●, respiratory depth (one animal). Temp. 20° C.

DISCUSSION

The results presented in Figs. 6–9 show that CO_2 in the inspired air influences respiration markedly at all percentages used. At percentages lower than 3 % CO_2 causes an increased pulmonary ventilation, while higher percentages cause a decrease in pulmonary ventilation to subnormal values. Higher CO_2 percentages (above 3–4 %) cause further transitory disturbances in the respiratory pattern ('periodic inhibition') by causing a 'Cheyne-Stokes-like' respiration.

Babak (1914*a*, *b*), v. Saalfeld (1934*a*), Vos (1936), and Boelaert (1941) found 'dyspnoea' at low (< 5 % CO_2) and 'inhibition' at higher CO_2 percentages. But most of their experiments on the influence of CO_2 on respiration lie outside the interval where CO_2 stimulates the *pulmonary ventilation*. Siefert (1896) used 100 % CO_2; the narcotic effect therefore was dominant in his experiments. Randall *et al.* (1944) found that, after a period of apnoea, respiration was stimulated by CO_2 even in very high percentages. This may be due to the fact that their CO_2 experiments lasted only until the appearance of the first groups of vigorous respirations after the apnoeic period (at the most 8 min.). Thereafter they shifted to room air again. This could, perhaps, give the false impression that the pulmonary ventilation is high even in the early period of CO_2 breathing where the respiratory frequency is reduced.

The present study shows that an increase in the CO_2 percentage in the inspired air causes an increase in respiratory depth (Fig. 9). The gradual increase in respiratory depth after the beginning of CO_2 breathing (Fig. 7) may imply that the respiratory depth follows the pCO_2 changes of the blood. It is presumed that the pCO_2 increases gradually from the beginning of the CO_2 breathing and, after some time, reaches a steady level. On shifting from CO_2 breathing back to room air breathing, the respiratory depth again decreases slowly (as opposed to the immediate change in frequency). This might also correspond to the presumably slow fall in the pCO_2 of the blood caused by the gradual CO_2 release from the tissues on returning to room air breathing. However, the respiratory depth did not increase when the pCO_2 of the blood was increased by raising the temperature (metabolic rate) of the animal. It is also possible that the increased respiratory depth may have been influenced by an oxygen lack in the respiratory centre, this oxygen lack being produced by the long apnoeic pauses after the start of the CO_2 breathing and maintained by the low respiratory rate in the steady state. This assumption, however, needs special investigation.

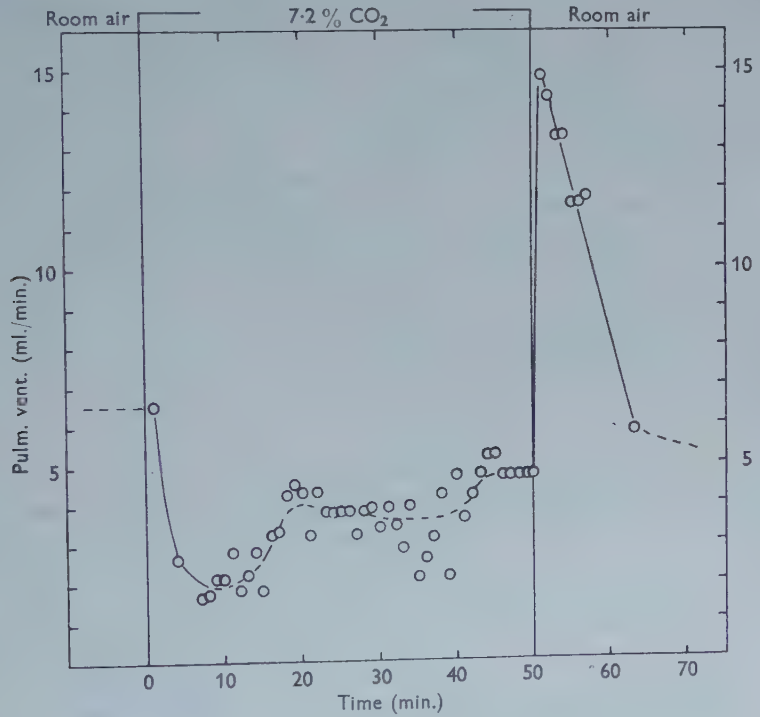

Fig. 8. As Fig. 7 (see Fig. 7), but showing the variation in pulmonary ventilation.

As for the respiratory frequency, no simple correlation seems to exist between frequency and blood pCO_2. At the beginning of CO_2 breathing and in the steady state, the respiratory frequency is the lower the higher the pCO_2 is in the blood.

An increase in pCO_2 produced by an increase in metabolic rate, however, increases the respiratory frequency (see discussion in Part II). It seems, then, that an increased blood and tissue pCO_2, produced by an increase in CO_2 content in the inspired air, causes the frequency to decrease, while an increased CO_2 tension in the tissues, when the CO_2 content in the inspired air is low, causes the frequency to increase.

The steady-state relationship between frequency and CO_2 content in the inspired air can be explained by the assumption that the respiratory frequency is depressed via chemoreceptive nerve endings in the lungs. An increase in activity of these chemoreceptors might occur as a response to increasing CO_2 content in the lungs. Experiments of v. Saalfeld, Vos and Boelaert have shown that such chemoreceptors exist and that the receptors must be situated in the lungs, not in the upper respiratory pathways. Thus the 'periodic inhibition' at the beginning of CO_2 breathing with higher CO_2 percentage is thought to be a reflex (Babak, v. Saalfeld, Vos and Boelaert). Boelaert (1941) concluded that inhibitory impulses from the chemoreceptors in the

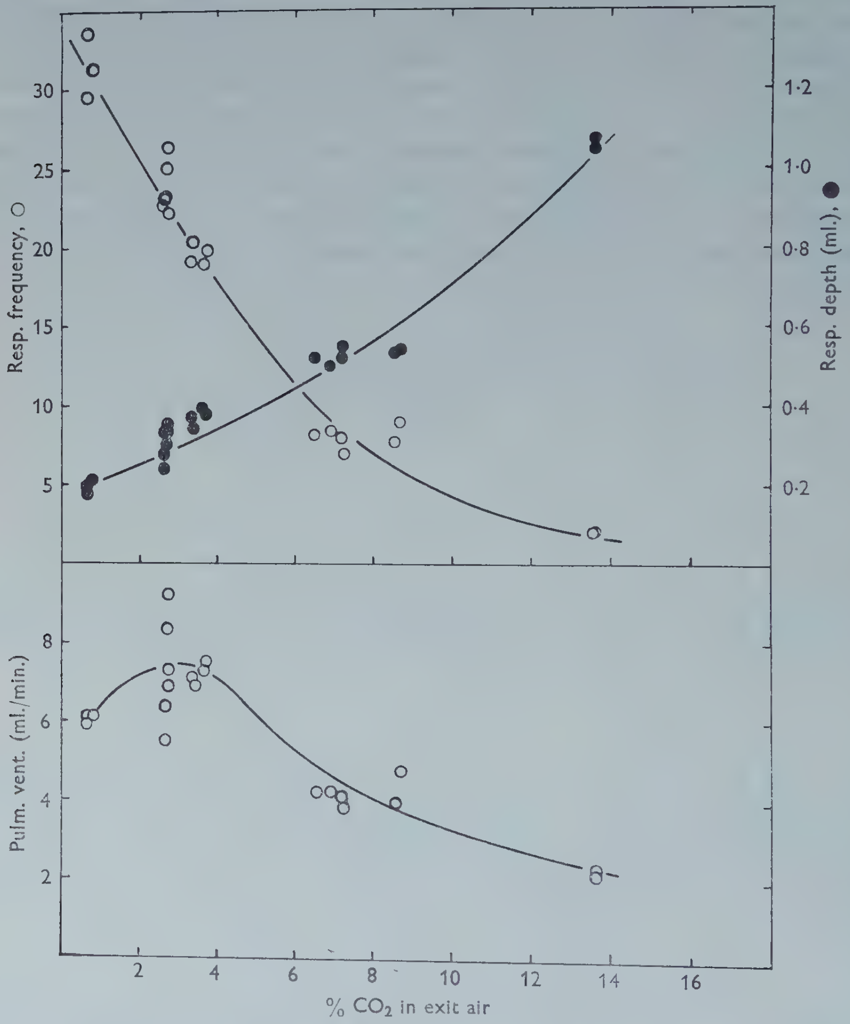

Fig. 9. Respiratory frequency, depth, and pulmonary ventilation in relation to the CO_2 percentage in the exit air. Above: ○, respiratory frequency; ●, respiratory depth. Below: ○, pulmonary ventilation (one animal). Temp. 20° C.

lungs reach the respiratory centre via the vagus nerve, as he found that the inhibition (depression of the frequency) was abolished when the vagus was cut. The causes of the return of the 'Cheyne-Stokes' respiration during prolonged CO_2 breathing to a regular pattern may be thought to be due to an adaptation of the postulated pulmonary chemoreceptors to the CO_2 stimulus, whereas the sudden increase in respiratory frequency, on shifting from CO_2 breathing to room air breathing, would then be due to the disappearance of the depression as room air enters the lungs. The respiratory frequency in the first few minutes after the shift is *higher than the normal* (about twice the normal steady state frequency). This may be an effect of the still high blood and tissue pCO_2 on the respiratory centre. As in the experiments with increased body temperature the CO_2 content in the inspired air is now low and an increased tissue pCO_2 seems to increase the respiratory frequency as shown in Part II.

In the steady state of CO_2 breathing, the inhibitory effect of CO_2 via the lung receptors may veil this direct accelerating effect that blood and tissue CO_2 seems to have on the frequency. However, no correlation between the previous CO_2 percentage and the maximum value of respiratory frequency after the shift to room air was found in the present experiments.

As for the pulmonary ventilation, this study seems to show that in *Lacerta* the pulmonary ventilation is not primarily regulated by CO_2. In the steady state the ventilation could, at the most, only be doubled by CO_2 administration. This small ventilatory increase (the result of the CO_2 effect on the respiratory frequency) is especially striking when the great ability of the animals to increase both respiratory frequency and depth is considered. According to Babak, a combination of low O_2 concentration and CO_2 in the inspired air gives a heavy 'dyspnöe' in *Iguana*. In man, Nielsen & Smith (1951) similarly have shown that, during hypoxia, the effect of CO_2 on the pulmonary ventilation is much increased. It seems possible that in the case of *Lacerta* also, a combination of CO_2 and low oxygen percentages can stimulate the pulmonary ventilation to much higher values than CO_2 administration alone.

SUMMARY

1. In two species of *Lacerta* (*L. viridis* and *L. sicula*) the effects on respiration of body temperature (changes in metabolic rate) and of CO_2 added to the inspired air were studied.

2. Pulmonary ventilation increases when body temperature increases. The increase is brought about by an increase in respiratory frequency. No relationship is found between respiratory depth and temperature.

3. The rise in ventilation is provoked by the needs of metabolism and is not established for temperature regulating purposes (in the temperature interval 10°–35° C.).

4. The ventilation per litre O_2 consumed has a high numerical value (about 75, compared to about 20 in man). It varies with the body temperature and demonstrates that the inspired air is better utilized at the higher temperatures.

5. Pulmonary ventilation increases with increasing CO_2 percentages in the inspired air between 0 and 3%. At further increases in the CO_2 percentage (3–13·5%) it decreases again.

6. At each CO_2 percentage the pulmonary ventilation reaches a steady state after some time (10–60 min.) and is then unchanged over prolonged periods (1 hr.).

7. The respiratory frequency in the steady state decreases with increasing CO_2 percentages. The respiratory depth in the steady state increases with increasing CO_2 percentages. This effect of CO_2 breathing is not influenced by a change in body temperature from 20° to 30° C.

8. Respiration is periodically inhibited by CO_2 percentages above 4%. This inhibition, causing a Cheyne–Stokes-like respiration, ceases after a certain time, proportional to the CO_2 percentage (1 hr. at 8–13% CO_2), and respiration becomes regular (steady state). Shift to room air breathing causes an instantaneous increase in frequency to well above the normal value followed by a gradual decrease to normal values.

9. The nature of the CO_2 effect on respiratory frequency and respiratory depth is discussed, considering both chemoreceptor and humoral mechanisms.

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SODIUM AND POTASSIUM FLUXES IN THE ABDOMINAL NERVE CORD OF THE COCKROACH, *PERIPLANETA AMERICANA* L.

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(*Received* 19 *October* 1960)

INTRODUCTION

The experiments of Hoyle (1953) on the effects of potassium on the electrical behaviour of locust peripheral nerve led to the suggestion that the continuous sheath which surrounded the nervous system of this insect functioned as a diffusion barrier restricting the entry of potassium ions into the underlying nerve cells. The subsequent investigation of Twarog & Roeder (1956) on the abdominal nerve cord of the cockroach confirmed the observations of Hoyle and showed that the presence of the cellular and fibrous sheath, or perilemma, apparently protected the central nervous system of this insect from the adverse effects of fluctuations in the ionic content of the haemolymph. It was also found that the perilemma restricted the entry of acetylcholine molecules into the central nervous system.

The presence of a relatively impermeable diffusion barrier around the central nervous system made it difficult to appreciate how the necessary exchanges of nutritive and excretory substances could take place between the haemolymph and the nervous tissues. In an investigation on the metabolism of some ^{14}C-labelled compounds in the abdominal nerve cord of *Periplaneta americana* L. it was found, nevertheless, that there was a rather rapid influx of the relatively large glucose and trehalose molecules through the perilemma (Treherne, 1960). In an attempt to resolve this apparently paradoxical situation the present investigation was undertaken to determine the rates of influx of sodium and potassium ions into the abdominal nerve cord of *P. americana* L.

In this paper the nomenclature of Scharrer (1939) has been used to describe the layers in the sheath of the cockroach central nervous system. Accordingly the sheath, or perilemma, is divided into two parts: the outer fibrous neural lamella and the inner cellular perineurium.

METHODS

The adult male *P. americana* L. was used exclusively in these experiments as it possesses a nerve cord which is relatively free from associated fat body.

The penetration of sodium and potassium ions into the abdominal nerve cord was studied after the injection of ^{24}Na and ^{42}K into the haemolymph of this insect. The radioactive ions were incorporated, as NaCl and KCl, in an experimental solution in which the major chemical components approximated to those of cockroach haemolymph (Table 3). To obtain a satisfactory level of activity in the nervous tissue it was necessary to inject 100 μl. of the radioactive solution which represented an appreciable increase in haemolymph volume. Experiments in which smaller volumes were injected did not, however, differ significantly from those in which 100 μl. were used and it is assumed that the increase in haemolymph volume did not affect the validity of these results.

In each experiment at varying times after the injection of ^{24}Na and ^{42}K individual cockroaches were decapitated and the abdominal nerve cord was removed by dissection from the dorsal surface. The isolated radioactive nerve cords were quickly washed in isotonic dextrose solution, dried on filter-paper and then weighed on a torsion balance. Haemolymph samples were collected using silicone-lined glass pipettes. The radioactivity in the haemolymph and tissue was determined using an end-window counter (Mullard MX 123).

The sodium and potassium concentrations of the nerve cord and haemolymph were measured by means of an EEL flame photometer, the tissue samples being previously ashed in a muffle furnace on pieces of platinum foil at temperatures of 460–480° C.

The freezing-point depressions of haemolymph and experimental solutions were measured by the method of Ramsay (1949) as modified by Ramsay & Brown (1955).

RESULTS

The measurements on the sodium and potassium concentrations of the haemolymph and the abdominal nerve cord are tabulated in Table 1. The results for the tissue concentrations are, despite the similarity in age, sex and diet of the individuals used, characterized by a rather high degree of individual variation. These data are similar in this respect to those obtained by Tobias (1948) with this tissue. The mean values for the nerve cord tissue were also of the same order as those given by Tobias, while the concentrations in the haemolymph were similar to the values of 164·2 mM./l. for sodium and 7·4 mM./l. for potassium obtained by Asperen & Esch (1956).

Table 1. *Concentration of sodium and potassium in the haemolymph and abdominal nerve cord of adult male cockroaches*

Tissue	Sodium concentration (mM./l. ± S.D. (n))	Potassium concentration (mM./l. ± S.D. (n))
Haemolymph	157·4 ± 28·0 (15)	12·3 ± 2·0 (15)
Nerve cord	103·2 ± 23·7 (14)	180·2 ± 26·1 (14)

The freezing-point depression of the haemolymph of adult males was also measured, the data being summarized in Table 2.

To inject the ^{24}Na and the ^{42}K into the haemolymph an experimental solution was devised in which the concentrations of the various salts and organic substances

Table 2. *The freezing-point depression of haemolymph from adult male cockroaches*

Serial	Δ° C.	Mean
1	0·960	0·897
2	0·890	
3	0·880	
4	0·895	
5	0·860	

approximated to those of the haemolymph. The substances incorporated in this solution are tabulated in Table 3. The sodium and potassium concentrations used in this solution were the same as those given in Table 1, the concentrations of the other ions being based on the values given by Asperen & Esch (1956) for cockroach haemolymph. The concentrations of trehalose and glucose were taken from the data of Treherne (1960). The total osmotic pressure of the solution was adjusted by the addition of the three amino-compounds, which were found by two-dimensional paper chromatography to be present in relatively large amounts in the haemolymph of this insect. The freezing-point depression of this fluid was 0·89° C. which approximated to the value of 0·897° C. for the haemolymph.

Table 3. *The composition of the experimental solution used in this investigation*

Substance	Concentration (mM./l.)	Substance	Concentration (mM./l.)
Na	157·0	Trehalose	36·9
K	12·3	Glucose	2·2
Ca	4·5	Glycine	30·0
Mg	4·0	Glutamic acid	35·0
Cl	184·1	Glutamine	30·0
H_2PO_4	0·1	Δ	0·89° C.
HCO_3	2·1		

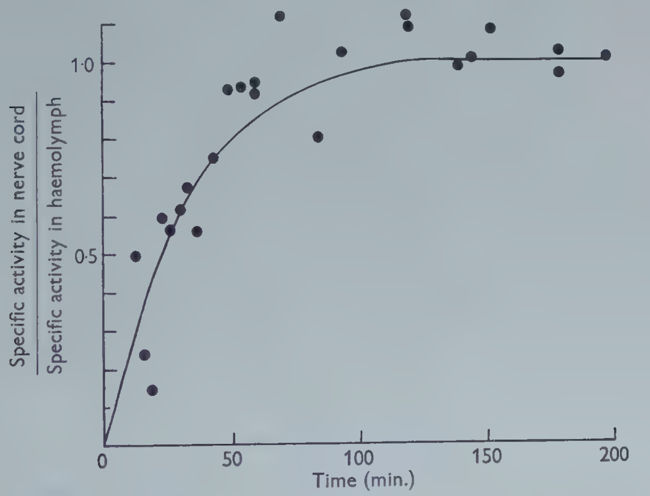

Fig. 1. The increase in specific activity of potassium in the nerve cord, relative to that in the haemolymph, following the injection of ^{42}K into the haemolymph.

The penetration of injected ^{42}K into the abdominal nerve cord from the haemolymph is illustrated in Fig. 1, in which the specific activity in the nerve cord relative to that in the haemolymph is plotted with respect to time. These results showed that the time for half-exchange of the nerve cord potassium with the ions in the haemolymph was approximately 24 min.

To interpret the results illustrated in Fig. 1 it is necessary to calculate the rate of influx of potassium ions into the nerve cord. Such a calculation in this *in vivo* system would involve a knowledge of the changes in the specific activity of the haemolymph potassium following injection. This decline in specific activity, which would tend to be minimized by the large volume of fluid injected, was unfortunately obscured by the large individual variation. Thus, following Shaw (1958), these influxes have been very approximately calculated using equations describing the exchange of ions between isolated living cells and the surrounding media (cf. Harris & Burn, 1949):

$$m_i = -\frac{1}{t} c_i \ln\left(1 - \frac{a_{in}}{a_{out}}\right), \tag{1}$$

where m_i = inward ionic flux, t = time, c_i = concentration of ion in nerve cord, a_{in} and a_{out} = specific activities in the nerve cord and haemolymph respectively.

Fig. 2 illustrates the semi-logarithmic plot of $c_i \ln(1 - [a_{in}/a_{out}])$ with respect to time. The line drawn through the points is the average of the angles formed by the lines joining the individual readings to the origin (Treherne, 1954). From the slope of the line shown in Fig. 2 it can be calculated, using equation (1), that the influx of potassium ions was equivalent to 312 mM./l. nerve cord water/hr.

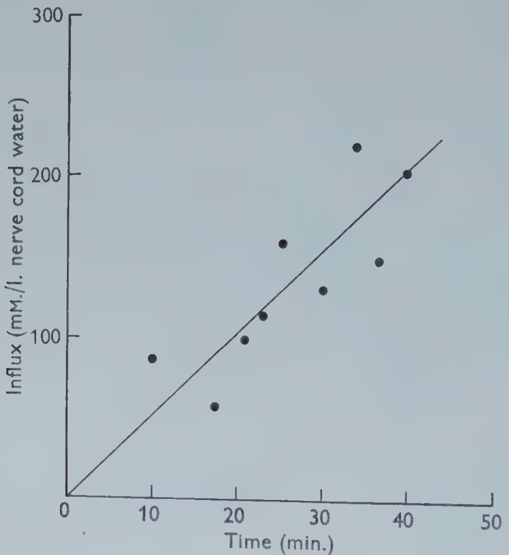

Fig. 2. The calculated influx of potassium ions into the nerve cord derived from the data in Fig. 1.

The entry of the injected ^{24}Na from the haemolymph is represented in Fig. 3 in which the ratio of the specific activities in the nerve cord and the haemolymph are plotted with respect to time. The data plotted as $c_i \ln(1 - [a_{in}/a_{out}])$ against time is illustrated in Fig. 4. From the slope of the calculated line drawn through the points it is possible to estimate that the influx of sodium ions was approximately 320·0 mM./l. nerve cord water/hr.

The sodium and potassium contents of the haemolymph and the nerve cord from the individuals used in these experiments are summarized in Table 4.

Because of the great differences in the concentrations of sodium and potassium in the haemolymph it is necessary to convert the calculated influxes for these ions to

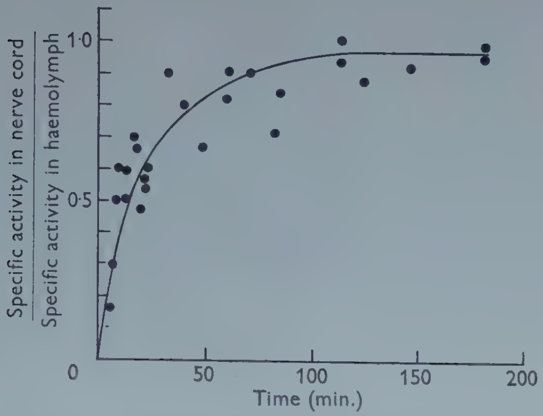

Fig. 3. The increase in specific activity of sodium in the abdominal nerve cord, relative to that in the haemolymph, following the injection of ^{24}Na into the haemolymph.

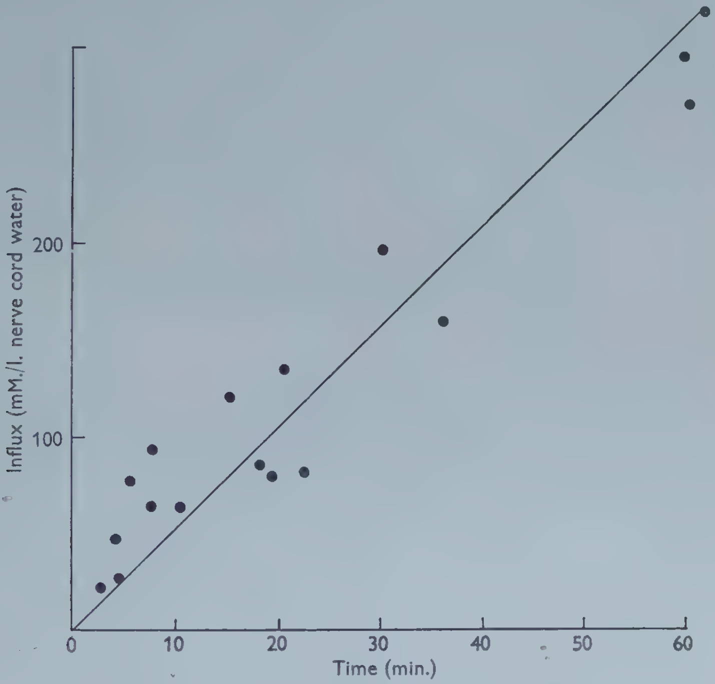

Fig. 4. The influx of sodium ions calculated from the data illustrated in Fig. 3.

Table 4. *Concentration of sodium and potassium ions in the haemolymph and abdominal nerve cord of individuals used in* ^{24}Na *and* ^{42}K *uptake experiments*

Tissue	Sodium concentration (mM./l. ± S.D. (*n*))	Potassium concentration (mM./l. ± S.D. (*n*))
Haemolymph	132·4 ± 20·7 (26)	9·2 ± 3·2 (24)
Nerve cord	98·3 ± 19·2 (26)	182·3 ± 22·8 (24)

transfer constants in order to compare their relative rates of penetration. A transfer constant can be related to the influx by the equation

$$k_{\text{in}} = \frac{m_i}{c_o}, \tag{2}$$

where c_o is the concentration of the ion in the haemolymph and k_{in} is the transfer constant in the direction in → out. k_{in} is thus the flux per unit outside concentration, having the units 1/time (hr.$^{-1}$). This constant, k_{in}, is not comparable with the k of Keynes & Lewis (1951), which is equivalent in this case to k_{out}. The values for k_{in} derived from the calculated influxes then become:

$$\textit{sodium}\ k_{\text{in}} = \frac{320 \cdot 0}{132 \cdot 4} = 2 \cdot 41\ \text{hr.}^{-1},$$

$$\textit{potassium}\ k_{\text{in}} = \frac{312 \cdot 0}{9 \cdot 2} = 33 \cdot 9\ \text{hr.}^{-1}.$$

To calculate the flux per unit area of nerve cord surface it is necessary to know the volume per unit area of this organ. In order to obtain an approximate idea of this ratio some isolated abdominal nerve cords were placed in liquid paraffin and their dimensions measured with a travelling microscope. The area and volume of the connectives were calculated by considering them as cylindrical structures. The values for the individual ganglia were estimated by considering these structures as oblate or prolate spheroids. By these rather crude methods the surface/volume ratio was estimated to be roughly about 6·4 cm.$^{-1}$. The influx per unit area of nerve cord surface can thus be represented as:

$$\textit{sodium}\ 13 \cdot 9 \times 10^{-12}\ \text{M cm.}^{-2}\ \text{sec.}^{-1},$$

$$\textit{potassium}\ 13 \cdot 5 \times 10^{-12}\ \text{M cm.}^{-2}\ \text{sec.}^{-1}.$$

DISCUSSION

The results outlined above indicate that there is a relatively rapid exchange of sodium and potassium ions between the abdominal nerve cord and the haemolymph in this insect. In fact the movements of the labelled potassium ions through the membrane surrounding the central nervous system in this insect were not far removed from those which have been measured in individual cephalopod and crustacean axons. In the nerve cord the estimated influx of potassium ions of roughly 13×10^{-12} M cm.$^{-2}$ sec.$^{-1}$ approached the value of $16 \cdot 7 \times 10^{-12}$ M cm.$^{-2}$ sec.$^{-1}$ obtained for single resting *Sepia* axons by Keynes (1951). These figures are more or less directly comparable for the outside concentrations of potassium were rather similar in the two cases, being 9·2 mM./l. in the cockroach haemolymph and 9·7 mM./l. in the artificial sea water used by Keynes. The movement of ^{24}Na into the cockroach abdominal nerve cord, approximately equivalent to $13 \cdot 9 \times 10^{-12}$ M cm.$^{-2}$ sec.$^{-1}$, was rather less than in the isolated *Sepia* axons where the measured influx across the resting membrane was $61 \cdot 0 \times 10^{-12}$ M cm.$^{-2}$ sec.$^{-1}$. In this case, however, the concentration of 458 mM./l. Na^+ in the artificial sea water employed in the *Sepia* preparations exceeded that in the cockroach haemolymph by almost three times; thus it seems that the sodium movements through the perilemma were of a similar order of magnitude to those for the cephalopod axons.

Comparison of the transfer constants for the entry of the ions into the nerve cord, where k_{in} for sodium was 2·4 hr.$^{-1}$ and for potassium was 33·9 hr.$^{-1}$, indicate that the movements of the individual potassium ions occurred about fourteen times more rapidly than those of the sodium ions.

The rapidity of these ionic movements through the perilemma are perhaps rather surprising in view of the observations by Hoyle (1953) and Twarog & Roeder (1956) that the membrane surrounding the nervous system apparently serves a protective function enabling the underlying nervous elements to function despite wide variations in the ionic composition of the surrounding media. These authors showed that in *Locusta* nerve fibres, and in the abdominal nerve cord of *Periplaneta*, removal of the continuous sheath associated with these structures resulted in a rapid interference with nervous function in the presence of excess potassium ions. The perilemma of the cockroach nerve cord seems to be less efficient in this respect than the nerve sheath demonstrated by Hoyle in the locust, for the *Locusta* nerve resisted block for as long as 4 hr. in a solution containing 140 mM./l. K^+ as compared with the cockroach nerve cord which became blocked in 22–30 min. in 140 mM./l. K^+ and in 12–18 min. in 180 mM./l. K^+. These experiments seem to have led to the general assumption that the sheath was functioning as a diffusion barrier restricting the entry of ions into the underlying nervous tissues. The possibility certainly exists that the membrane bounding the central nervous system may be impermeable to net ionic movements, the influxes measured in this investigation being largely due to some sort of exchange diffusion mechanism of the type postulated by Ussing (1949). However, Tobias (1948) has shown that the potassium content of the cockroach nerve cord increased rapidly following a rise in the level of the haemolymph potassium, from an average of 27·1 mM./l. to 49·2 mM./l., produced by the ingestion of 1·4 N-KCl. In view of the fairly massive net movements of potassium into the nerve cord demonstrated by Tobias it must be concluded that the sort of impermeability postulated above does not play an important part in this system. The present experiments clearly suggest then that a dynamic steady state rather than a static impermeability must exist across the perilemma in this insect. The important observation by Hoyle that impairment of the tracheal supply reduced the efficiency of the perilemma may be a reflexion of this dynamic state, for if the membrane functioned merely as a static diffusion barrier then it might not be expected to be closely linked to the metabolism of the system.

It has been often assumed in the past that the selective permeability of the perilemma was due to the outer fibrous sheath, the neural lamella. It is difficult to visualize how such a membrane could function to maintain a dynamic equilibrium between the haemolymph and the central nervous system. The observations of Twarog & Roeder (1956) who found that silver nitrate penetrated the fibrous layer, and of Wigglesworth (1960) who found that the neural lamella was apparently freely permeable to dye molecules, suggest that it is the cellular perineurium which is effective in regulating the fluxes of ions and molecules across the perilemma.

These experiments showed that the greater part of the sodium and the potassium within the central nervous system exchanged completely and rapidly with the labelled ions in the haemolymph, for in both cases the specific activities within the nerve approached 1·0. Thus there is no evidence in this system for any appreciable amounts of very slowly exchanging sodium or potassium such as have been postulated to occur in nerve by Rothenberg (1950).

The results outlined here do not, of course, throw much light on the nature of the processes involved in the transfer of ions between the haemolymph and the central nervous system in this insect. It is hoped that in future investigations some of the factors associated with these ionic transfers may be elucidated.

SUMMARY

1. The influx of sodium and potassium ions into the central nervous system of *Periplaneta americana* has been studied by measuring the increase in radioactivity within the abdominal nerve cord following the injection of ^{24}Na and ^{42}K into the haemolymph.

2. The calculated influx of sodium ions was approximately 320 mM./l. of nerve cord water/hr. and of potassium ions was 312 mM./l. of nerve cord water/hr. These values are very approximately equivalent to an influx per unit area of nerve cord surface of $13{\cdot}9 \times 10^{-12}$ M cm.$^{-2}$ sec.$^{-1}$ for sodium and $13{\cdot}5 \times 10^{-12}$ M cm.$^{-2}$ sec.$^{-1}$ for potassium ions.

3. The relatively rapid influxes of these ions are discussed in relation to the postulated function of the nerve sheath as a diffusion barrier. It is suggested that a dynamic steady state rather than a static impermeability must exist across the sheath surrounding the central nervous system in this insect.

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A NEW SYSTEM FOR CONTINUOUS RESPIROMETRY OF SMALL AIR-BREATHING INVERTEBRATES UNDER NEAR-NATURAL CONDITIONS

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INTRODUCTION

The limitations of conventional respirometers, confined to short-term experiments and working under constant temperature conditions, have recently become manifest in two main fields. First, the investigation of short-term responses of organisms to temperature changes, as carried out by Grainger (1956) and others, has shown the existence of oscillatory changes in respiration and revealed the need for a respirometer in which animals can be subjected to temperature changes. Secondly, ecologists, in an effort to place food-chain studies on a quantitative basis, have turned to respiration rate as a generally applicable measure of metabolic activity regardless of the energy source of the species concerned (Balogh & Gere, 1953; Bornebusch, 1930; Gere, 1957; Kalle, 1948; Lindemann, 1942; Macfadyen, 1948, 1957; Overgaard, 1949; Teal, 1957). In this field of work it is of less interest to determine instantaneous values of respiration than to be able to relate the parameters, such as mean and excursion, of a daily cycle of respiration rate to those of the daily temperature régime. This represents a radical departure from the conventional analytical study of respiration rate, and demands a new technique appropriate to the study of respiration under near-natural conditions. The apparatus to be described has been evolved to meet these requirements and provides a continuous record of respiration under conditions of fluctuating temperature.

PRINCIPLES OF OPERATION

The respirometer to be described incorporates two principles which are well known. The first is that introduced by Barcroft of uniting the two halves of a differential respirometer by a block of brass in order to equalize the temperatures in the two halves and at the same time to isolate the system from the surrounding atmosphere and thus render it independent of barometric fluctuations. The second principle is the electrolytic generation of oxygen to replace that removed by the animal, thus permitting longer-term experiments than would otherwise be possible. This appears to have been arrived at independently on a number of occasions (Fernandes, 1923; De Boer, 1929; Swaby & Passey, 1953; Capraro, 1953) and has been used in conjunction with the Warburg constant-volume respirometer. Swaby & Passey introduced the further principle of causing the contraction of gas volume, which results from respiration and the absorption of carbon dioxide in alkali, to draw up sulphuric acid to a platinum anode inside the experimental chamber and thus making the electrolytic oxygen generation automatically equal to the demands of the experimental material. Oxygen was measured indirectly in a gas voltameter where the hydrogen liberated from the cathode was collected.

In a Barcroft-type respirometer, such as that described here, sulphuric acid cannot be used as the electrolyte, because the hydrogen liberated at the cathode will increase the pressure inside the isolated system; but by using copper sulphate solution metallic copper of negligible volume is deposited at the cathode and by using electrical methods of current measurement the gas voltameter can be dispensed with.

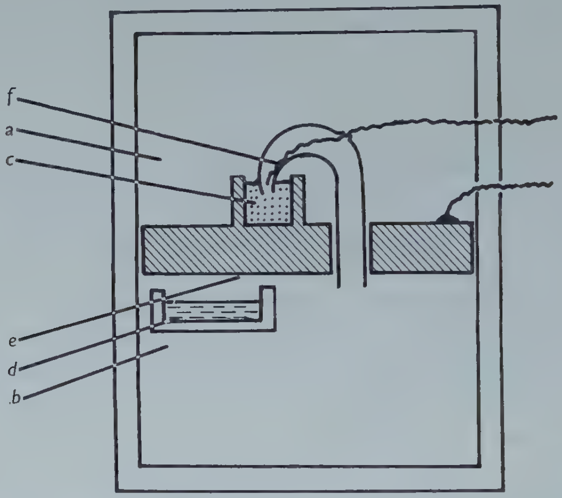

Fig. 1. Simplified diagram of respirometer chamber working with direct current only. *a*, Compensating chamber; *b*, animal chamber; *c*, copper sulphate vessel; *d*, alkali; *e*, metal partition; *f*, positive lead for oxygen generation.

A simple automatic respirometer can thus be constructed on the lines shown in Fig. 1. The space inside a sealed glass vessel is divided into two by a metal partition and the only communication is through a glass capillary containing a platinum electrode. The metal serves as a cathode and the upper or compensating chamber contains copper sulphate solution into which the capillary dips.

Uptake of oxygen by the animal and absorption of carbon dioxide by the alkali create a negative pressure in the animal chamber. Copper sulphate enters the capillary and touches the platinum anode. Oxygen is generated until the pressure forces the copper sulphate solution down and breaks the circuit. Oxygen is thus supplied intermittently, when demanded by the animal. Oxygen production can be measured by a silver voltameter (Macfadyen, 1956) or by various types of electrical recorder.

A simple respirometer of this type was described by the author (1955) but at the order of accuracy required for small soil arthropods and for use as a continuous recorder (as distinct from merely integrating the oxygen generation over long periods of time) it proved unsuccessful, mainly on account of the formation of bubbles of gas in the electrolyte at the anode. If surface tension is low, bubbles are formed and the current fluctuates very greatly; no simple means of making an accurate continuous record could be devised. If anti-foaming agents are added, operation is jerky and the results are inaccurate. For larger animals and greater rates of respiration an all-d.c. respirometer on these lines is quite feasible, as has recently been shown by Wintringham (1959), who, however, does not use a compensating chamber.

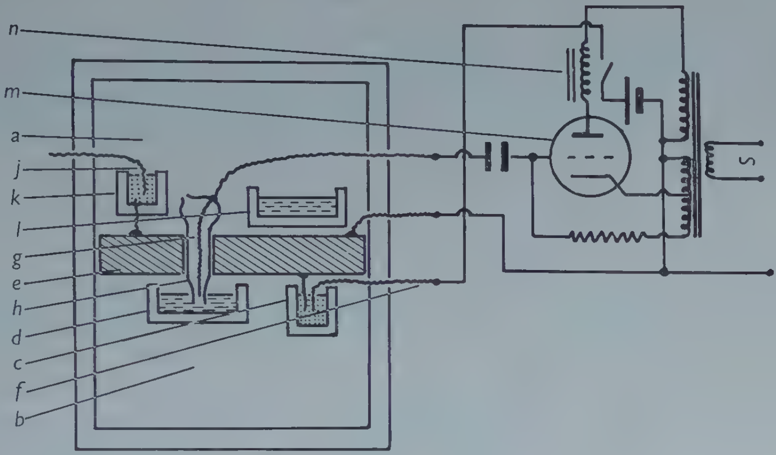

Fig. 2. Simplified diagram of respirometer chamber working with separate pressure-detecting and oxygen-generating electrodes. *a–f*, As in Fig. 1; *g*, lead to pressure-detecting electrode; *h*, pressure-detecting electrode; *j*, lead to compensating oxygen generator; *k*, compensating copper sulphate vessel; *l*, compensating alkali vessel; *m*, triode amplifier; *n*, relay for switching on d.c. to *c*.

A more accurate and sensitive respirometer can be constructed by separating the two functions of oxygen generation and pressure-change detection at two different electrodes as, for example, in Fig. 2. The only change introduced here is that pressure change is detected by an electrode which is connected in a bridge circuit supplied with alternating current. By using a thermionic valve as detector very low currents are passed, polarization of the electrode is prevented and small changes in the level of the meniscus can be detected. Such a change results in the valve relay closing and switching on direct current to the anode in the copper sulphate solution in the animal chamber. It will be noticed that in this version the compensating chamber contains duplicate vessels for both alkali and copper sulphate. These are necessary to prevent spurious gas-volume changes when temperature changes are made (see below). They are also important at the start of an experiment because carbon dioxide in the compensating chamber is absorbed at the same rate as in the experimental chamber; the two oxygen generators can be used in turn to bring the meniscus in the pressure-detecting electrode to its working position.

With the basic layout of Fig. 2, and using a vessel of 25 cm.^{3} capacity in each half, sufficient accuracy can be achieved for measuring respiration rates of small arthropods of the order of 1 mg. weight. In order to increase accuracy various other principles were investigated, including those advocated by Burk & Hobby (1954) in their hydrostatic amplifier respirometer and 'paradox' respirometer. These attempts failed to increase sensitivity possibly because the principles which they employ cannot be applied to the Barcroft-type respirometer. The increased sensitivity needed for small species down to about 0·01 mg. weight is obtained instead by the use of scaled-down versions of the respirometer herein described, together with improved methods of pressure-change detection.

A PRACTICAL A.C. RESPIROMETER

The various elaborations of the arrangement illustrated in Fig. 2 which are required to produce a workable instrument can be considered under two main headings: the design of the respirometer vessel itself and that of the control and recording system. The various component parts of these will now be considered in turn.

(*a*) *The design of the respirometer chamber* (Fig. 3)

The effects of temperature change

In order that temperature changes shall not produce different rates of expansion in the animal and compensating chambers the following principles must be observed in practice:

(1) The two chambers should be as similar as possible in size, shape and construction so that rates of heating of both gases and liquids will be the same. If the volumes and surface areas of liquids are different, transient differences in vapour pressure will occur as the temperature changes. The magnitude of such differences is correlated more closely with the acceleration than with the velocity of temperature change.

(2) The chambers must be thermally united by a good conductor and made independent of room temperatures by insulation or by submergence in a water-bath. Thick glass walls used in earlier models were found to be inadequate as insulators when used in air in a 'constant temperature' (i.e. ± 1° C.) room. Since electrically conducting leads must pass into the chambers these leads must be as long and thin as possible and arranged symmetrically.

Spurious gas absorption is an important factor to be guarded against in the design of the chambers. This can occur at rates much greater than the respiration rates being measured, due to (1) the use of water which is not fully aerated (e.g. recently distilled water) in making up solutions, and (2) chemical oxidation of metal when in contact with dilute alkali. The remedy for the first trouble is obvious. The second has been overcome by the use of titanium or copper, plated first with silver and then with rhodium, for all metal parts.

The strength of the solutions used in the chambers is determined by two factors: the environmental demands of the animals, and the balance of vapour pressures. Saturated copper sulphate solution is in equilibrium with air at about 98 % relative humidity and so is 4 % alkali, so that no distillation between these solutions occurs. Furthermore, most terrestrial invertebrates thrive in humidities of this order. If drier conditions are required and stronger alkali is used the surface of the copper sulphate chamber must be made as small as possible. If the respirometer were used with aquatic animals, by placing them in dishes of water inside the chamber, the vapour pressure balance might be upset and the thermal capacity of the animal chamber would certainly be increased: both these effects could presumably be balanced by a similar dish in the compensating chamber.

The low level of carbon dioxide in the air results in somewhat artificial conditions for the animals, as in most other respirometers. It is proposed to rectify this by the use of carbon dioxide buffers (Umbreit, 1957), but these have not yet been tried.

The pressure-detecting system is simply a switch which causes oxygen generation to be started or stopped with a minimal change in pressure. In principle a non-conducting capillary coated with a thin layer of a noble metal could be used but no fully satisfactory coating has yet been produced. A Pyrex glass capillary with a fine platinum electrode fused in the glass has been found to be chemically and mechanically reliable. At first the wire was carefully centred in the capillary but this resulted in discontinuous operation. If the wire is arranged to touch the side (as in Fig. 8) a film of dilute alkali is stretched between the wire and the bulk of the fluid. The controller is sensitive to a

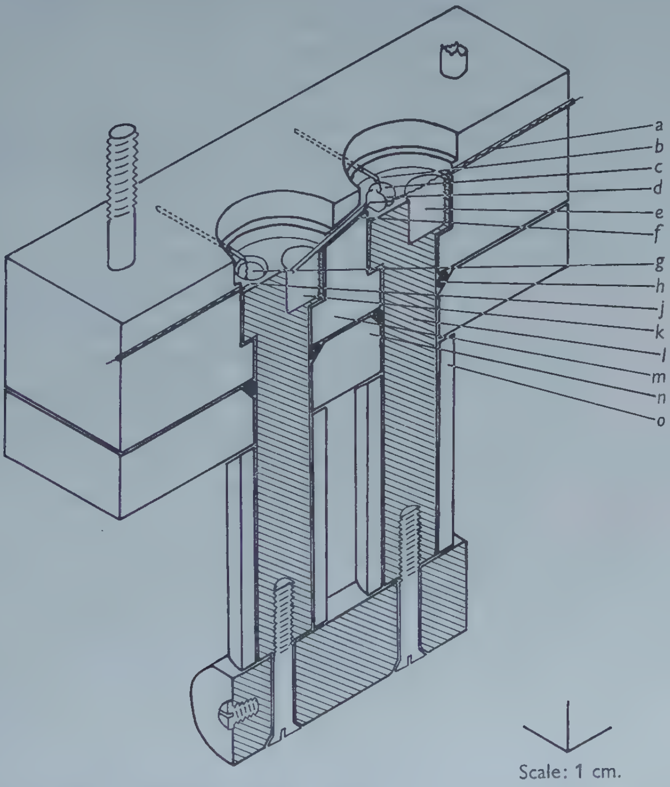

Fig. 3. Section through plastic type respirometer of 2·5 c.c. capacity. Animal chamber on the left, compensating chamber on the right. The remaining half is similar except that the small vessels *c* and *g* are balanced by similar ones containing alkali (for temperature compensation). The animals rest on a gauze or in a glass chamber (not shown) within the left-hand chamber. The lid (not shown) is held in place by wing nuts on three screws; these screws also hold in place the bottom piece *n*. The cut surfaces of the titanium (or rhodium-plated copper) bars are shaded; these are bolted to a common copper heat-sink which may be immersed in a water-bath. *a*, Lead to pressure-detecting electrode; *b*, oxygen-generating electrode, compensating chamber; *c*, oxygen-generating cell containing copper sulphate solution; *d*, glass bulb of pressure-detecting electrode; *e*, alkali vessel of compensating chamber; *f*, capillary tube of pressure-detecting electrode; *g*, oxygen-generating cell, animal chamber; *h*, synthetic rubber 'O'-ring; *j*, spare connexion for use with thermistor and included for heat balance; *k*, alkali vessel, animal chamber; *l*, titanium (or rhodium-plated copper) bar in the top of which vessels are drilled; *m*, main body of respirometer made from styrene/rubber (Styron 475); *n*, bottom plate of respirometer made from Perspex; *o*, insulating tubes of Perspex.

change of approximately 3000 Ω in 25,000 Ω so that the stretching and contraction of the film can cause the oxygen to be switched on and off without the film being broken or the column being sucked into the narrow region above the tip of the wire. Used in this way, operation of the oxygen generator results from barely perceptible changes of liquid level. There is no advantage to be gained from making the capillary diameter less than about 0·8 mm.

The use of plastics was approached very cautiously, especially in view of reports that these can be highly toxic (see Jeanson-Luusinang, 1958). However, the materials referred to were the earlier plastics such as polyvinyl chloride which contain large quantities of toxic 'plasticisers'. Polystyrene can be produced without the incorporation of plasticisers and an impact-resistant grade containing rubber (Styron 475) was found to have no effect on longevity of mites kept in a vessel lined with this material and to be well suited to the present purpose.

The plastic respirometer vessel has the following advantages:

(1) Almost perfect thermal symmetry. Transient spurious uptake rates of less than 0·2 mm.^{3}/hr. are produced by temperature changes of 1° C./hr. and accelerations of 5° C./hr.^{2}. These results could be further improved if necessary by adjustment of solutions in the two chambers.

(2) The actual vessels are not submerged in the water-bath, and animals can be observed during experiments without disturbance. They can also be given any desired light régime. It should be noticed, however, that the sensitivity of the apparatus is such that the thermal conduction of the $\frac{1}{2}$ in. Perspex lid cannot be neglected. A strong draught or a finger placed asymmetrically on the lid will cause an immediate spurious 'uptake' or 'generation' of gas.

(3) The vessels are robust and very easily loaded and emptied. They stay suspended above the water-bath, and connected to the controller in a normal room; they can be changed in 2 or 3 min. While the lid is being screwed down, the conductivity of the pressure-detecting electrode is monitored. If the meniscus is too low, the screw on the animal chamber side is screwed down until it rises and then that on the other side, so that the meniscus starts close to the working position. Final adjustment is made by generating oxygen alternately in the two chambers and, as a result, recordings can begin within a few minutes.

(4) Ease of construction. All the parts can be made without difficulty on a small lathe, and small (sensitive) vessels are no more difficult to make than larger ones. So far, vessels from 2·5 to 20 c.c. capacity have been made without difficulty.

(b) *The design of the control and recording system*

In principle this is as follows:

A timing mechanism, driven by a synchronous electric motor, 'interrogates' the respirometer at regular intervals, 'offering' oxygen. This process will be referred to as the cycle. If at the beginning of the cycle oxygen is required (as indicated by the position of the alkali meniscus in the capillary) the oxygen-generating current is switched on and continues to flow until the next 'interrogation' at the beginning of the next cycle. If the 'no more oxygen required' signal is given in the middle of an 'on' cycle (i.e. a cycle during which the oxygen-generating current is flowing) the current is not switched off until the 'interrogation' at the beginning of the next cycle; similarly, if the 'oxygen required' signal is given in the middle of an 'off' cycle the current is not switched on until the 'interrogation' at the beginning of the next cycle. Oxygen is thus supplied in units or 'doses'. Records are made by perforation of a moving strip of paper. On the time track a mark is made at the beginning of each cycle; on the respirometer track a mark is made at the beginning of each 'on' cycle. The respiratory rate is calculated from the mark/space ratio on the respirometer track, taken over some convenient number (usually 30) of cycles.

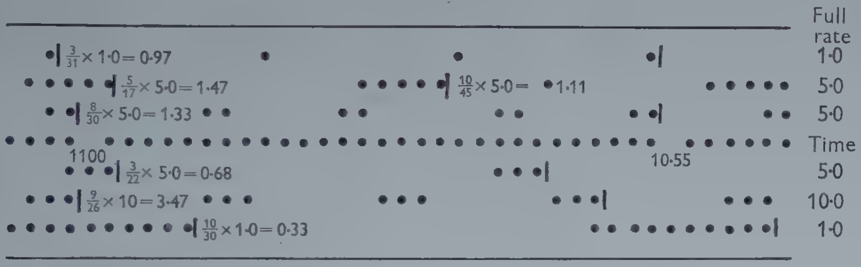
Full rate
$\frac{3}{31}\times 1{\cdot}0=0{\cdot}97$
$\frac{5}{17}\times 5{\cdot}0=1{\cdot}47$
$\frac{10}{45}\times 5{\cdot}0=$ 1·11
$\frac{8}{30}\times 5{\cdot}0=1{\cdot}33$
1100
10·55
$\frac{3}{22}\times 5{\cdot}0=0{\cdot}68$
$\frac{9}{26}\times 10=3{\cdot}47$
$\frac{10}{30}\times 1{\cdot}0=0{\cdot}33$
1·0
5·0
5·0
Time
5·0
10·0
1·0

Fig. 4*a*. Copy of a record of a respiration experiment marked to show the manner of calculating respiration rates. The pre-set rates at which oxygen would be generated if switched on continuously are shown on the right.

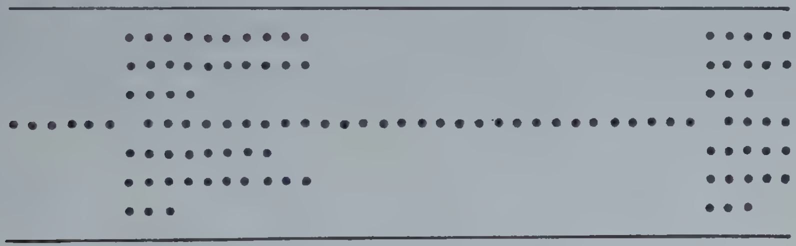

Fig. 4*b*. The same record as 4*a* with 'batching' circuits in operation (see p. 339).

In this system the control and recording of six respirometers involves three separate pieces of equipment, the full details of which are given in Appendix 2 (Figs. 9, 10). They are briefly:

(*a*) An amplifier enabling the signal corresponding to the position of the meniscus in the pressure-detecting electrode to open or close an electromagnetic relay; the circuit for this differs from that in Fig. 2 mainly in that a pentode amplifier is used and that the switching of the oxygen-generating direct current is over-ridden by the timing mechanism so that closing and opening of the relay can only occur at set times. Various switches and controls are provided so that the current can be controlled and metered.

(*b*) A timing mechanism. This involves a switch, driven by a synchronous motor, and a number of relays so arranged as to govern the operations of the oxygen-generating cells in the respirometers and to send signals to the recorder in order to wind on the paper and make a mark when the appropriate cell has received a supply of oxygen.

(*c*) A recorder. This consists of a solenoid-operated drive for the paper and seven solenoid-operated markers equipped with steel gramophone needle tips which prick the paper. Six of the markers correspond to the six respirometers and produce marks every time oxygen is provided. The seventh provides a time track which is marked at the beginning of each cycle, i.e. every time oxygen is 'offered'. This track is interrupted every 30 cycles by a cam-operated switch coupled to the paper drive; the resulting gaps at about 3 in. intervals facilitate the reading of the record. An example of an actual record is produced in Fig. 4*a*, in which the time track is central and the 30-cycle gaps represent ends of 5 min. intervals. The record can be read in many different ways but the following method is usually adopted.

(1) The oxygen supply current of each respirometer is set to a certain rate such that, if oxygen were continuously demanded, the rate of supply would be a whole number of cubic millimetres per hour. (The meter of Fig. 9 is directly calibrated in these units at a number of fixed temperatures.) The rate chosen is about twice the maximum anticipated demand so that oxygen will be accepted for about 50% of the time.

(2) The ratio of occasions when oxygen is accepted to the total number when it is offered (30) can be measured for each 5 min. period and the ratio multiplied by the chosen rate (item 1).

(3) However, strict adherence to this rule may result in an apparent periodicity in respiration rate since 'acceptances' may occur in groups which succeed one another at intervals of more or less than 5 min. It is therefore preferable to modify the method of reading. A reading period is arbitrarily considered to start with the first of a group of marks and to end with the last of a group of spaces. During this period the ratio of marks to marks plus spaces (not necessarily 30) is multiplied by the rate factor and is recorded for the corresponding 5 min. period. The record in Fig. 4*a* has been marked with time of day and fractions as read. The reading process is facilitated by the use of a ruler calibrated to fit the punch marks.

PERFORMANCE

A. *Performance at constant temperature*

(i) *Sensitivity*. A limit to performance is set by the volume-sensitivity of the respirometer which is in turn determined by (*a*) the size of the chambers, and (*b*) the sensitivity of the pressure-detecting system. With 2·5 c.c. chambers the change in volume required to turn the oxygen supply on or off is of the order of 0·1 mm.^{3}.

The size of the respiratory chamber is set by the size of the animal. The sensitivity of the pressure-detecting system depends upon the diameter of the capillary and upon the amplification factor; in both these directions there is scope for improvement if this becomes necessary.

(ii) *Stability*. Within the limits of sensitivity stability is complete. That is to say, if the respirometer is set up without any respiring tissue and with the capillary meniscus in the working position it will remain so indefinitely.

(iii) *Accuracy*. The record shows the number of 'doses' of oxygen supplied in a given time, and the accuracy of the method in terms of rate of oxygen consumption depends upon the accuracy with which the amount of oxygen supplied in a 'dose' is known. The electrochemical equivalent of oxygen is of course known with a high degree of accuracy. The evolution of oxygen from copper sulphate solution at the low rates here used has been tested in a modified Warburg apparatus and no departure from theoretical expectation has been found. The accuracy is therefore determined in practice by (*a*) the stability of the oxygen-generating current, (*b*) the accuracy with which it is measured, (*c*) the extent of electrical leakage in the associated circuits and (*d*) the accuracy with which time is measured. The following steps have been taken to account for these factors:

(*a*) The use of a large-capacity 6 V. dry battery, partially discharged, as an independent source and the use of a high resistance in series with the copper sulphate cell. (*b*) The calibration of the good quality moving-coil meter using a Tinsley potentiometer and the avoidance of current values involving small deflexions. This is probably the weakest link in the chain at present and may limit accuracy to ±3% but a more accurate meter could be used if the nature of the experiments demanded it. (*c*) This is a matter of careful construction and regular checking of respirometers before use, but no serious trouble has been experienced in practice. (*d*) The synchronous motor running from 50 c./s. mains is unlikely to vary by more than 1%, but a clockwork movement could be substituted if necessary. There remains the possibility that although the timing mechanism itself may be accurate the operation of the switch gear may involve some delay and thus curtail the time during which the oxygen-generating current flows. As is explained in the Appendix, the time lost in this way is not more than 0·2 sec. (2% of a 10 sec. dose). This loss could be reduced if necessary in more accurate work.

As a check on the level of accuracy of the whole respirometer, comparative trials were carried out with a Warburg respirometer (Shandon with 15 c.c. reaction vessels). Disks of carrot root (6 mm. diameter × 2 mm. thick, weight about 8 mg.) were used. The mean respiration rates over a 4 hr. period were:

Warburg:	1·35, 1·02, 0·67, 1·19	mean 1·06 ± 0·29 mm.^{3}/hr./mg.
New respirometer:	1·00, 0·81, 1·29, 1·48, 1·15	mean 1·14 ± 0·26 mm.^{3}/hr./mg.

Similar results were obtained in other trials and it was concluded that there was no difference in absolute level of accuracy between the two respirometers.

Any method which involves measurement of amount of oxygen consumed in a finite time interval must inevitably lag upon events when the rate of oxygen consumption is changing. If the time interval is reduced the lag is reduced, but at the expense of accuracy. The errors thus occasioned can be calculated from the sensitivity of the respirometer and from the following parameters which can be varied at will so as to provide the most acceptable compromise: (*a*) the strength of the oxygen-generating current; (*b*) the duration of the cycle; (*c*) the number of cycles over which the rate of oxygen consumption is calculated.

B. *Performance under changing temperature*

The effect of change of temperature on a respirometer with a compensating chamber is to produce movements in the connecting capillary due to changes in gas expansion and vapour pressure in the two chambers. In a symmetrical apparatus such movements are transient and can usually be correlated with either the acceleration or the velocity of temperature change. An example of such an effect is shown in Fig. 5*a*. If temperature changes are not to produce effects of this kind, the two chambers must be connected by a good thermal conductor, and preferably some inertia in the form of high thermal capacity should be present in the system. The plastic type of respirometer contains a total of 400 g. of copper (thermal capacity about 40 cal./° C.) and the rate of conduction between each chamber and the water-bath is about 5000 times as great as that through the plastic and the leads. The surface areas of the fluids present in the depressions in the copper rods are independent of the volume, but if volumes are

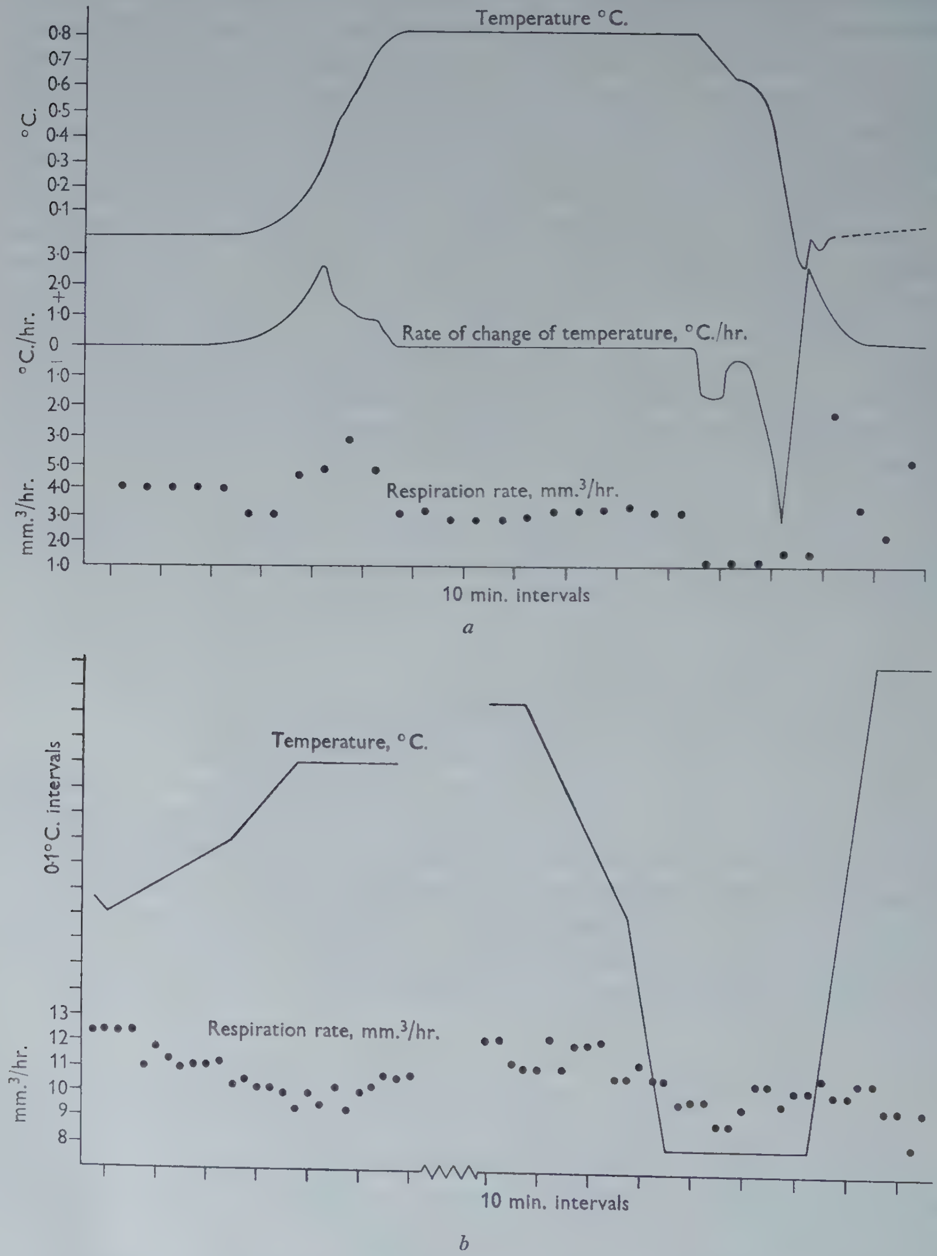

Fig. 5. Graphs to show spurious changes in respiration rate, produced by changes in temperature. (*a*) Temperature, rate of change of temperature and readings from earlier glass type of respirometer. (*b*) Temperature and readings from compensated plastic type of respirometer.

markedly unequal they affect the thermal inertia of the chambers in a predictable manner (the gas in the chamber containing least fluid expands faster as the temperature of the whole system is raised). For normal purposes the chambers may be filled with equal volumes of fluids as judged by eye, but when large rates of temperature change are to be made, preliminary experiments must be carried out in which the volumes are adjusted to produce a negligible spurious response. In this way rates of change of 5° C./hr. and accelerations of 25° C./hr.^{2} have been shown to produce spurious effects of less than 1 mm.^{3}/hr. and lasting for less than 10 min. (Fig. 5*b*). There is no reason to suppose that these results could not be improved on, but these figures correspond to faster changes of temperature than have been required up to the present.

RESULTS

Experiments to date have been confined to constant temperatures maintained to ±0·02° C. by a Sunvic thermostat, relay and proportionating head; but a thermostat giving sinusoidal temperature cycles of controlled amplitude has also been constructed and will be described in due course. Temperature can be measured in the water-bath or inside the respirometer by means of a thermistor. A number of results have been obtained with invertebrates of different sizes and, whereas some species show little diurnal variation in rate, others show marked changes, a phenomenon which is thought not to have been reported before in terrestrial invertebrates. Some typical results are given in Figs. 6 and 7.

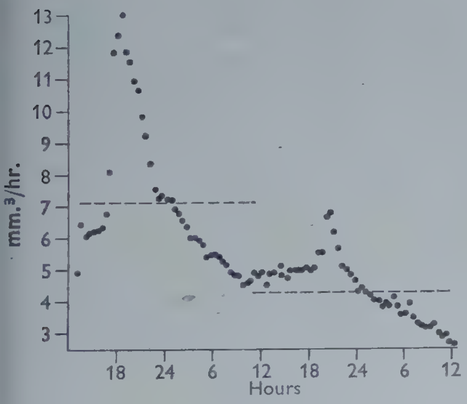

Fig. 6

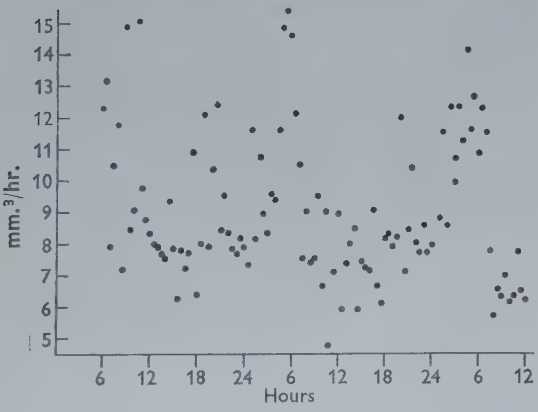

Fig. 7

Fig. 6. The first two complete diurnal cycles of respiration taken from a 4-day experiment on *Platybunus triangularis*, ♀ pre-adult. This experiment was exceptional in showing a rapid decrease in mean rate (at the end of 4 days the animal was alive but respired at only 0·2 mm.^{3}/ hr.). Temperature 19° C.

Fig. 7. The final 2½ days of a 5-day experiment on *Oniscus asellus* showing a tendency to high rates about dawn. Temperature 18° C.

Applications

The respirometer is intended for investigations in which long-term measurements at variable temperatures are required. In the case of water-living forms such as Protozoa and Nematoda the Cartesian diver respirometer (Holter, 1943; Linderstrøm-Lang, 1943) has greater sensitivity and gives consistent results (Overgaard, 1949). The more complex reactions of air-breathing animals and their dependence on diurnal temperature and light cycles for normal activity (Cloudsley-Thompson, 1951 *a*, *b*) demand the simulation of these cycles and the use of continuous recording during longer experiments. The study of acclimatization and reactions to sudden changes of temperature has only recently been attempted (Grainger, 1956; Kerkut & Taylor, 1956) and existing types of respirometer are unsuitable. It is hoped that in these two fields which are common to physiology and ecology the new apparatus may prove particularly useful. In addition, the recording facility may well prove of use in more conventional respirometry, especially where a number of experiments are to be run simultaneously. There is no reason to suppose that the limits of accuracy, sensitivity and independence of fluctuating temperature have yet been approached. The cost of the complete apparatus with six plastic chambers is certainly less than £50 for materials.

SUMMARY

1. A new system of respirometry for long-term experiments on small air-breathing invertebrates is described for use in work where changing temperature conditions are essential. There is no reason why the same system should not be used for larger animals.

2. The respirometer is of the Barcroft, compensating type, isolated from the atmosphere, and has provision for temperature equilibration in the two vessels.

3. Oxygen is automatically replenished by electrolysis of copper sulphate solution in the chamber as it is used by the animal and the rate of replenishment is recorded.

4. The main features in design of such an apparatus, its limitations and accuracy are discussed. The accuracy is comparable with that of a Warburg respirometer and the sensitivity, at better than 0·1 mm.^{3} of oxygen/hour, is about ten times as great.

5. A special control and recording system has been developed in order to reduce expense and simplify the interpretation of the data from six respirometers over long periods.

6. It is shown that the errors arising from temperature changes can be reduced to acceptable levels for most purposes. The apparatus is therefore suitable for use in the study of acclimatization and of responses to rhythmical temperature changes.

7. Examples of respiration records are given which illustrate the possibilities of being able to follow respiration rates through several diurnal cycles.

8. Practical details of construction are given in the appendices.

The need for a respirometer of this nature first became clear to me after discussions with Mr Charles Elton, F.R.S., in about 1951. It is thanks to his encouragement and the patient advice of my colleagues, both in the Bureau of Animal Population, Oxford, and in the Botany and Zoology Departments at Swansea, that the demands of a seemingly impossible specification have been met. An earlier draft of this paper was seen by Prof. V. B. Wigglesworth who made many suggestions and criticisms which have resulted in radical improvements both to the apparatus and to the paper. Imperial Chemical Industries, Waunarlwydd Titanium Plant, generously supplied titanium metal, and British Resin Products, Research and Development Division, kindly produced the polystyrene blocks. Messrs Edwards High Vacuum Ltd. gave advice on the use of 'O' rings, waxes, and silicone greases. Mr Tweedy, manager of South Wales Transport Co. Ltd., and his colleagues advised me on the use of T.I.M. bus ticket paper and arranged for a supply. To all these people I am duly grateful; and also to my wife whose encouragement and advice have been constant through many set-backs. During the first period of this work I was in receipt of a grant from the Nature Conservancy, to whom I am most thankful.

APPENDIX I

Construction of the plastic respirometer vessels (Fig. 3)

(*Note.* A list of suppliers of equipment and materials is given at the end of Appendix 2.)

The larger parts of the chambers are turned on a lathe, care being taken that the horizontal surfaces are quite flat. The metal parts are either of titanium metal (I.C.I. Metals) or of copper plated with silver and then rhodium (Gerhardy). No difference in performance with these two materials has been detected. The cost of the titanium would normally be higher, but it is possible that the plating might eventually deteriorate. The leads (of 36-gauge copper wire) are threaded through capillary of 1 mm. o.d. drawn from Pyrex glass and terminate in soldered joints to 42 s.w.g. platinum wire which is fused into the closed end of the capillary. This wire constitutes the electrodes in the oxygen-generating cells and must be so mounted as to avoid touching the sides of the cell. The pressure-detecting electrode is made from 42 s.w.g. platinum wire and 4 mm. o.d., 2·5 mm. i.d. Pyrex glass. The glass is cleaned with detergent, chromic acid and distilled water, and reduced to about 0·8 mm. internal diameter (measured under a microscope). The glass is blown into a small bulb (Fig. 8*a*), of 5 mm. diameter with the aid of a micro-burner as in the construction of Cartesian divers (Holter, 1943, p. 421), except that the walls are deliberately made much thicker. Two hollow 'horns' are then formed on the bulb and burst (Fig. 8*b*) and the platinum wire, previously fused into a bead, is placed so that the tip rests against the opposite wall of the capillary (Fig. 8*c*). The platinum wire is soldered to its lead after the electrode has been fitted. A capillary manometer is used to check the operation of the electrode. Great difficulty has

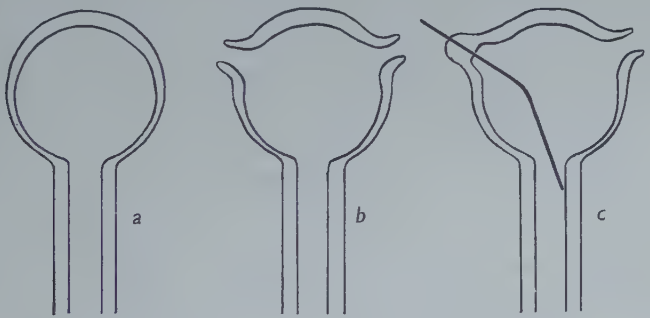

Fig. 8. Details of the construction of a pressure-detecting electrode from glass capillary and platinum wire.

been experienced in producing gas-tight joints which can stand thermal expansion and occasional wetting with alkali. The main joints are made with rubber 'O' rings (Edwards) and these, provided that the maker's specifications are followed, have proved completely gas-tight as well as permitting dismantling of the apparatus if necessary. The joints between the polystyrene block and the glass capillaries are made with a polystyrene cement (suggested to me by Mr Hayward and described by Lund, 1949), containing 15 g. polystyrene, 5 ml. dibutylphthalate and 30 ml. xylol. This cement is very reliable but it should be allowed at least 3 days to dry and to allow the plasticiser to evaporate. The junction between the lid and the body of the respirometer is sealed with Edwards High Vacuum Silicone Grease.

A variety of experimental chambers can be placed inside the respirometer according to need. Immobile materials can be placed on a coverslip cut to allow passage of air. Larger animals can rest on a piece of stainless steel gauze provided that faeces will not fall into the solutions below. Alternatively, simple cells can be made from hanging drop rings and coverslips. In experiments with carrot slices it was found that these become desiccated when placed on a gauze above 4% and even 1% alkali, but when one side rested on a piece of coverslip the rate of desiccation was very greatly reduced.

APPENDIX 2

The control apparatus (Fig. 9)

(1) *The amplifier.* This has a.c. supplied to both anode and grid so that when the two supplies are in phase, anode current flows; when in antiphase the valve does not conduct. The screen is supplied with 180 V. d.c. which was found empirically to give greatest sensitivity under these conditions. The transformer supplying the bridge in the grid circuit of the valve is connected so that, when the pressure detector is on open circuit, the grid is supplied with 18 V. in antiphase to the anode; as the impedance between the pressure-detecting electrode and the earth connexion falls below 28,000 Ω the phase is reversed, the valve conducts and the anode relay opens. The anode circuit resembles a normal power pack smoothed by resistance and condenser.

(2) *The timing mechanism* is governed by a self-starting synchronous electric clock motor (surplus stores) which closes a switch S_5 for about 3 sec. once in each cycle. A snap-action mechanism in this switch is essential to prevent multiple firing and sparking (Fig. 10). The motor drives a cam cut from brass which during most of the period rubs on the lower piece of beryllium copper; this piece is cemented with Araldite (Aero Resins Ltd.) to a piece of laminated resin plastic, and this in turn to the upper spring of beryllium copper which is the live contact of the switch. The cam is connected to earth by a slip-ring. Only when the spring has been fully raised does the tip of the cam make a snap connexion with the spring itself. The circuit is opened equally suddenly as the spring falls from the tip of the cam point.

Relay D is driven directly by S_5; one of the contacts applies a negative potential to the common point between relay A on the one hand and the 250μF. condenser in series with relay B on the other. As the condenser charges up on the closing (and discharges on the opening) of the clock switch S_5, relay B closes; otherwise it is open. The condenser discharge also maintains relay A open for a while after the clock switch has opened.

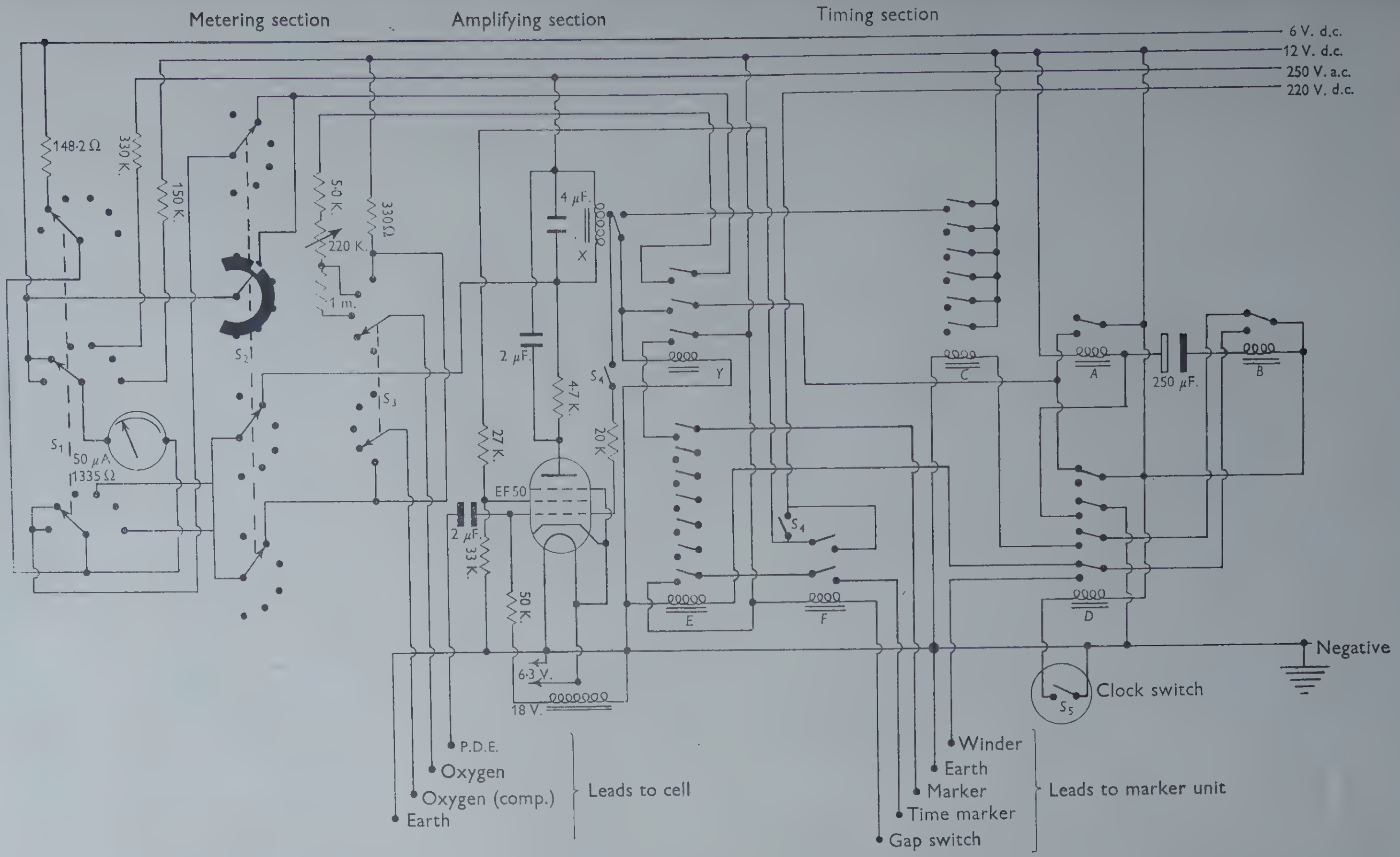

Fig. 9. Circuit diagram of the respirometer controller. Power supplies are omitted. The amplifier circuit and connexions are repeated six times. In all cases the contact arms of the relay move towards the relay coil when this is energized. For fuller description see text, Appendix 2.

By using suitable permutations of the opening and closing of the contacts on these relays the following sequence of operations is performed once in each cycle (see Fig. 11). (The phases of initial make, closure, initial break and opening of the clock switch S_5 will be numbered 1, 2, 3 and 4, respectively, in the description which follows; their duration is approximately 1, 2, 1 sec., and from 6 to 56 sec., respectively.)

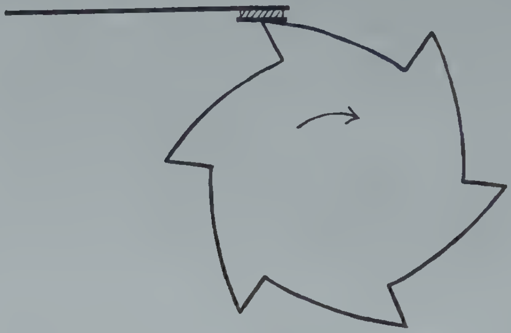

Fig. 10. Principle of snap-action mechanism in clock switch. The shaded part is insulation, joined to the beryllium copper strips with Araldite cement.

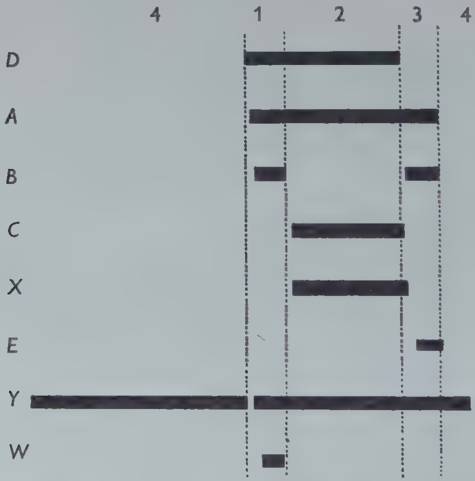

Fig. 11. Sequence of operations in the timing section of the controller. Black bars indicate the period during which the relay in question is energized. *A* to *E* are relays as shown in Fig. 9. The clock-switch S_5 closes at the same time as relay *D*. *X*, Supply to contact of relay *X* (and thence to relay *Y*) by way of relay *C*; *Y*, supply to relay *Y* through *D* (inverse) and *A* in parallel. *W*, Supply to paper-drive solenoid. The phases labelled 1 to 4 correspond to the movements of the clock switch S_5 and the charging of the 250 μF. condenser as follows: 1, S_5 closes, condenser charging. 1 sec. 2, S_5 remains closed, condenser is charged: 2 sec. 3, S_5 opens, condenser discharges. 1 sec. 4, S_5 remains open, condenser is discharged 6 sec. or 56 sec. according to time periodchosen.

(*a*) *In the recorder unit*

Phase 1: the paper-drive solenoid (connected to 'winder' terminal in Fig. 9) is energized through relay *D*.

Phase 3: all appropriate pens are energized through relay *E* (the corresponding hold-on relays *Y* having closed previously). The time-marker pen is energized every cycle except that once every 30 cycles the circuit is interrupted by a switch on the film drive unit so that a gap is left which facilitates reading the record.

(*b*) *In the amplifier circuit*

As explained previously the function of the timing mechanism is to ensure that oxygen is generated for definite periods of time. When respiration causes the grid resistance of the amplifier to rise and the anode relay (X) to close nothing happens until the end of the current phase (4). When the clock switch S_5 closes, first after a delay of one second relay C closes and then relay Y closes. The contacts on this relay perform three functions: (i) the coil of relay Y is supplied direct with d.c. and cannot then be opened during the ensuing period regardless of what happens to the anode relay; (ii) the circuit to the corresponding pen solenoid is completed so that, when relay E closes during phase 3, this pen will make a punch mark; and (iii) direct current is switched to the positive electrode in the copper sulphate cell in the respirometer, thus generating oxygen.

This state of affairs persists for the remainder of phase 4. During phase 1 of the next cycle (see Fig. 11) the direct positive supply to relay Y through its own contacts is interrupted—by relay D—for about 0·2 sec. only. If relay X at this time is still closed a further period of oxygen generation will follow, but if it has opened as a result of no more oxygen being required, relay Y will remain open for the ensuing period.

(*c*) *Additional facilities*

Temperature in the respirometer cells can be measured by thermistors whose resistance can be recorded by any suitable recording milliammeter. An inexpensive device for this purpose has been described (Kempson & Macfadyen, 1954) in which the position of a microammeter needle is recorded on film. The timing and control sections of the present apparatus can be substituted for those of the temperature recorder so that only the camera and circuit selector of the recorder need be used. Alternatively, the thermistors can be connected directly to a bridge circuit metered by a high-resistance recording milliammeter such as the Fielden servograph; this has been done and it provides a very accurate and easily read record but is limited to reading four thermistors only. Again, if mean temperatures only are required a temperature integrator (Macfadyen, 1956) can be used.

Batching mechanism. At the cost of some loss of sensitivity the reading of the paper record can be made easier by batching all the marks for a given set of 30 cycles at the beginning of the set. This has the effect of making the paper read like a series of histograms as in Fig. 5*b*. The anode relay is made to lock itself open as soon as any gap in oxygen demand occurs and to stay open until the end of the set of 30 cycles. At the end of the set the relay is allowed to close and the pent-up demand for oxygen is then satisfied during the next set of cycles. The locking-open is done by connecting the otherwise unused contact on the X relay to the grid circuit of the same valve; interruption of the screen current to all valves by relay F restores the relay X to a closed position. This device is best used with a cycle of 10 sec. duration and even then the mean length of the 'off' period is increased to $2\frac{1}{2}$ min. with a corresponding decrease in sensitivity. A switch (S_4) is provided to maintain the screen supply and cut out the bacthing mechanism when not required.

(3) *The metering and switching section.* Switch S_1 provides facilities for (*a*) metering the voltage drop across the anode relays of the six amplifiers by switching and thus checking on the correct operation of these circuits, (*b*) for inserting the 4 in., 50 microampère meter in the oxygen-generating circuits in order to set the rate of oxygen supply. The accuracy of this meter is not very high, and for more careful work than has been attempted so far a better class instrument could be used. Two ranges are provided covering 1–11 and 10–100 mm.^{3}/hr. oxygen rates. The face of the meter is directly calibrated in these units at 10°, 15° and 20° C. respirometer temperature. An additional meter position provides for a check on the continuity of the oxygen-generating circuits.

S_2 selects from the six circuits that circuit whose operation is being monitored by S_1.

S_3 is repeated for each circuit and is used to select a fast or slow rate of oxygen generation (further controlled by the 220 K resistor). Further positions are used to apply a 'force' voltage (at 30 mA.) to either of the oxygen generators during initial setting up so as to bring the liquid in the pressure-detecting electrode to its working position.

(4) *Components.* Most components used in the control unit were derived from government surplus sources or were bought from Radiospares Ltd. The heavy-duty relay *E* (from Londex Ltd.) handles the solenoid currents, the remaining relays are of Post Office type, all with 200 Ω coils except the relays *X* (5000 Ω) and relay *B* (500 Ω). Relays *Y* have heavy-duty contacts. Condensers are of paper block type except the two high values which are electrolytic. Resistances are of standard carbon type except for the 220,000 Ω variable controls which are of the pre-set wire wound type (Egen Electric Ltd.).

(5) *The marker unit.* The mechanical details of this unit will depend on available materials; the main points of design concern the paper wind and the solenoids. Bus ticket paper 1·5 in. (38 mm.) wide was chosen as a cheap recording medium (6 rolls at 2*d.* each are used in a week with cycles of 10 sec. duration), but it is not so easy to use as perforated film which can be driven by a sprocket wheel and is more rigid. Difficulty was experienced in achieving consistent spacing and in preventing sideways movement and creasing of the paper. These faults can be corrected by arranging for the paper to pass between rollers, of which one is driven by a solenoid and ratchet mechanism and the other (of small diameter) is freely floating in guides but mounted on a spindle which is pulled by strong springs towards the spindle of the driven roller. The unused roll of paper should rest in a box whose sides help to guide the paper towards the rollers. The marking needles are mounted on rocking arms whose other ends are pulled downwards by springs and upwards by solenoids (Phillips Control). These are of a type (41 12½ 12 DC) intended for intermittent use on 12 V. d.c. and provide ample force for the purpose. In fact, it is advisable to connect them in series with a 2 Ω resistance to reduce the penetration of the needle and this may usefully take the form of a Post Office counter with parallel resistance.

List of suppliers

Aero Research Ltd., Duxford, Cambridge.
British Resin Products, Research and Development Division, Barry, Glamorgan.
Edwards High Vacuum Ltd., Manor Royal, Crawley, Sussex.

List of suppliers (cont.)

Egen Electric Ltd., Charfleet Estate, Canvey Island, Essex.
Fielden Electronics Ltd., Wythenshawe, Manchester 22.
I.C.I. Metals Division, Titanium Plant, Waunarlwydd, Swansea, Glamorgan.
Johnson Mathey and Co. Ltd., 78 Hatton Gardens, London, E.C. 1.
Londex Ltd., 207 Anerley Road, London, S.E. 20.
National Cash Register Co., North Circular Road, London, N.W. 2.
Phillips Control (G.B.) Ltd., Farnborough, Hants.

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SOME OBSERVATIONS ON THE SWIMMING BEHAVIOUR OF THE ANEMONE *STOMPHIA COCCINEA*

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INTRODUCTION

Sea anemones are a group of animals whose diagnostic structural features are remarkably constant. Their diverse behaviour patterns can nevertheless be related to anatomical differences, especially in the distribution of muscular system and nerve-net. The arrangement of the nervous system and sense cells is less readily detected than that of muscles, for these elements are diffuse and require special staining methods for recognition. As yet their structure is known in detail only in *Metridium* (Pantin, 1952; Batham, 1956; Batham, Pantin & Robson, 1960), and variations in other anemones corresponding to observed physiological differences would not be unexpected.

Properties of the neuromuscular and sensory systems can be analysed most readily in rapid responses to external stimuli, such as the reflex closure of the sphincter in *Calliactis* (Pantin, 1935; Passano & Pantin, 1955). Inherent slow activity, on the other hand, which has been demonstrated in *Metridium* (Batham & Pantin, 1950*b*, *c*) and in *Calliactis* (Needler & Ross, 1958) has yet to be interpreted in terms of sensory and nervous activity.

Responses vary unexpectedly from one species to another. The swimming behaviour of *Stomphia coccinea* is a response to contact with certain starfish and could not be predicted from what is known of other anemones. The unusual circumstances of this behaviour were described by Yentsch & Pierce (1955), and Sund (1958) and Wilson (1959) have contributed more recent studies. In the present paper this behaviour pattern is examined further, and an attempt made to interpret the sequence of activity in underlying structural components. It may later be possible to relate various special features more satisfactorily to what is known of the behaviour of other actinians.

MATERIAL AND METHODS

Stomphia coccinea belongs to the deep-water family Actinostolidae, and has been described by Carlgren (1893, 1921) and Stephenson (1935). It ranges from Siberia through Scandinavia to the Pacific, and is infratidal in relatively cold waters, having been recorded between 9 and 445 m. (Stephenson, 1935).

The present observations were carried out at the University of Washington Marine Laboratories at Friday Harbor. Anemones were usually obtained from President Channel at a depth of about 200 m., where the summer temperature of the water probably does not attain 12° C. and considerable bottom currents result from tidal changes Many specimens were found on shells of *Modiolus modiolus*, and they were kept in running sea water at 10–15° C. and fed occasionally. *Dermasterias imbricata*, one of the starfish which causes the anemone to swim, is a Northern Pacific species which may be collected intertidally. It did not occur in dredge hauls containing *Stomphia*.

A kymograph and a Kodak Cine Special II were used for recording. Details of methods are given in relevant sections.

SEQUENCE OF BEHAVIOUR

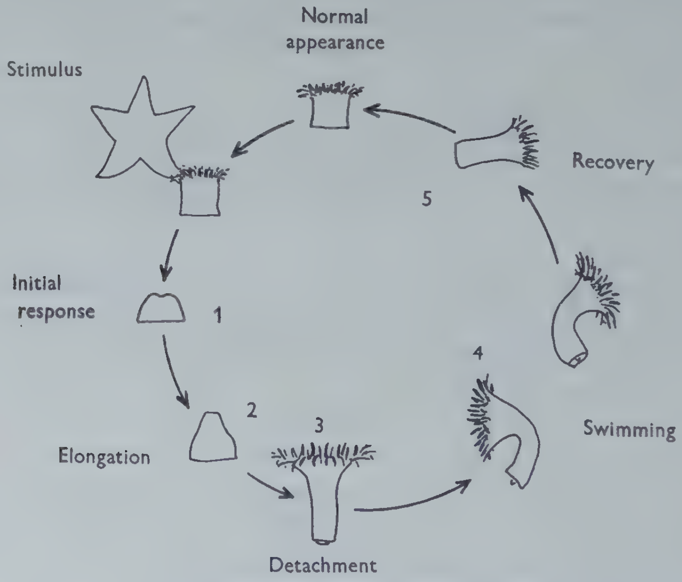
The general pattern of swimming behaviour in *Stomphia* has been described by Yentsch & Pierce (1955), Sund (1958), Wilson (1959), and Hoyle (1960). It is summarized here and in Text-fig. 1 for reference in the subsequent analysis.

Stimulus. Surface of the starfish *Dermasterias imbricata* in brief contact with the tentacles (up to 10 sec.). *Hippasteria spinosa*, the other effective starfish (Sund, 1958), was not available.

(1) *Initial response.* Tentacles adhere to the starfish on contact. After several seconds they contract, followed at once by contraction of the sphincter and retractor muscles.

(2) *Elongation.* As the sphincter relaxes, a wave of elongation passes down the column. The tentacles and disk reappear and expand to their fullest extent.

(3) *Detachment.* At the same time the foot is released from the substratum, and unless it is very firmly attached it decreases in diameter. The centre of the pedal disk, initially convex, becomes delimited by a deepening circular groove, and is constricted to a conical projection.



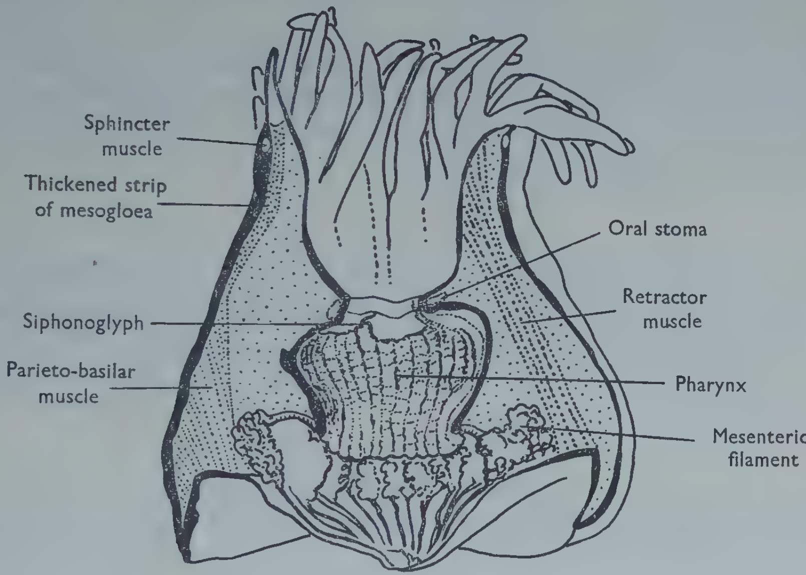
Text-fig. 1. Main features of swimming sequence.

(4) *Swimming.* A series of abrupt bending movements of the column ensues, which may cause the animal to 'swim'. Although the frequency of the contractions falls off, they may continue for several minutes. The bending movements may be preceded by a vigorous whirling of the column about its base.

(5) *Recovery.* An inactive period follows, during which the anemone resumes its normal shape. During this time it may not respond to renewed stimulation. The surface of the pedal disk presently exhibits a marked facility of adhesion, and as it re-attaches to the substratum the ordinary sessile position is regained.

ANATOMICAL STRUCTURE

The following account of the anatomy of *Stomphia* agrees with descriptions given by Carlgren (1893, 1921) and Stephenson (1935), and refers to parts of the animal which will be discussed later. Several features can be correlated with the swimming behaviour.



Text-fig. 2. Diagram of *Stomphia coccinea* in vertical section (cf. Pl. 1*a*).

The general plan of the anemone is shown in Pl. 1 and Text-fig. 2. There are no cinclides, and the only opening to the coelenteron is the mouth. None of the anemones examined was found to have a pore in the centre of the pedal disk like that shown by one of Yentsch & Pierce's specimens.

Below the tentacles is a strong mesogloeal sphincter muscle (Text-fig. 2, Pl. 2*a*). Although continuous with the endodermal circular muscle of the column, which is also well developed, the sphincter is clearly defined and can often be distinguished in living specimens by the presence of a pink pigment in the muscle fibres. This could be an actinohaeme such as occurs in certain other deep-water anthozoans like *Tealia* (Fox & Pantin, 1944). The circular muscle continues into the foot, oral disk and tentacles.

As seen in Pl. 1*d*, there are usually eighteen pairs of perfect mesenteries. Six primary and twelve secondary pairs may be distinguished by the corresponding arrangement of tentacles. Incomplete mesenteries develop progressively all the features of complete mesenteries, and the youngest cycles show the unequal development of a pair which characterizes the Actinostolidae (see Pl. 1*d*, *e*). None of the mesenteries reaches the centre of the pedal disk, which is thus free (Pl. 1*a*, *e*).

The perfect mesenteries carry three different muscles. The strong longitudinal retractors are endocoelic except in the directive mesenteries, and resemble those of other anemones (Text-fig. 2, Pls. 1*b*, 2*b*). On the opposite face there are transverse fibres of the radial muscle. In addition, there is on this surface a well-developed parieto-basilar muscle, which originates along the base of the mesentery. It is seen in Pl. 1*b* as a triangular area inserted below the tentacles about two-thirds the way up the column. As the parieto-basilar muscle tapers towards the upper part of the column, the mesogloea of the septum between it and the sphincter is thickened into a substantial ridge which might almost be regarded as a tendon (Text-fig. 2, Pl. 1*c*). The parieto-basilar muscle develops as a fold which grows over the radial muscle fibres on the same side of the mesentery. The relative positions of these two muscles and of the retractor are shown in Pl. 2*b*.

In the pedal disk, endodermal basilar muscles run on each side of a mesentery at its junction with the foot. The radial muscle of the oral disk, on the other hand, and the longitudinal muscle of the tentacles, are ectodermal. As in certain other anemones, however, they are sunk into the mesogloea.

THE INITIAL RESPONSE

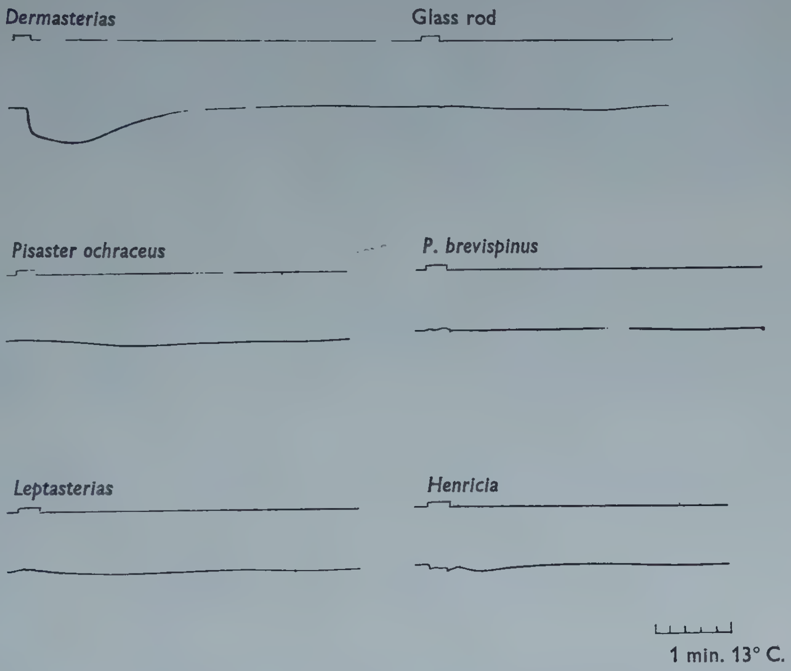
The first part of the response was studied in preparations of the sphincter with the crown of tentacles intact. These were prepared from anemones anaesthetized for half an hour with equal parts of $7\frac{1}{2}\%$ $MgCl_2 . 6H_2O$ and sea water (Batham & Pantin, 1951), and left in running sea water for 24–36 hr. before use. Experiments were carried out at temperatures between 9° and 12° C.

Nature of the stimulus

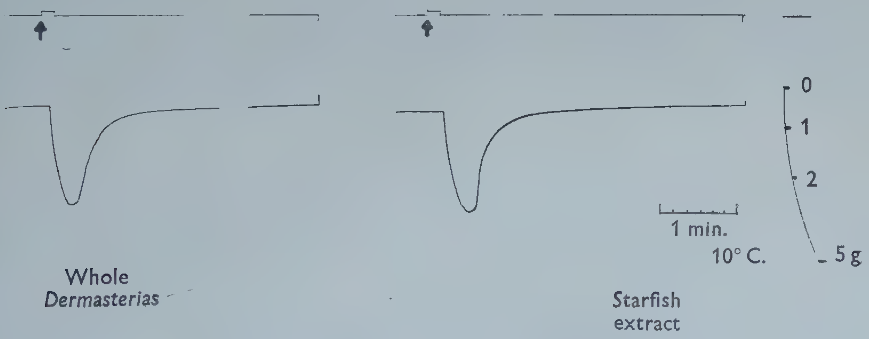
The specific quality of the sensory stimulus provided by starfish which release swimming behaviour has already been pointed out (Yentsch & Pierce, 1955; Sund, 1958). Sund made numerous laboratory and field tests which established that only two out of many local starfish, and none of the common invertebrates found in the same locality as *Stomphia*, would cause swimming. Preliminary work with *Dermasterias imbricata* and *Hippasteria spinosa*, both of which provoke a response, suggested that some parts of the starfish, in particular the aboral surface, were more effective than others. Further analysis of the stimulus provided by starfish is in progress (Ward, 1958, and current work).

When the tentacles are stimulated by the aboral surface of *Dermasterias*, they adhere to the starfish, and after a latent period of at least 5 sec., both tentacles and sphincter contract. Control tests such as rubbing the tentacles with a glass rod, and contact with various other starfish, cause no reaction. Records from an isolated sphincter preparation are shown in Text-fig. 3. The initial sphincter reflex thus indicates the specificity of the whole response. The stimulus cannot be a purely mechanical one, and must have a chemical component to which the tentacles are sensitive.

From work in progress (Mr J. Ward, which he kindly allows me to quote), it is known that an extract prepared by homogenizing the aboral surface of *Dermasterias* in sea water releases swimming in *Stomphia* as effectively as whole starfish. Such an extract applied near the tentacles of sphincter preparations causes a contraction like that produced by contact with starfish (Text-fig. 4). The extract was freshly prepared according to Ward's method, by homogenizing 1 g. of aboral epidermis in 3 ml. sea water for 5 min. and centrifuging. A few drops of the clear supernatant would cause an anemone to swim vigorously. In testing sphincter preparations, 0·5 or 1 ml. were delivered about 1 cm. away from the tentacles with a syringe. After a test, the sea water was changed twice and the preparation allowed to recover for 30 min. This confirms that the sphincter response can be evoked by a purely chemical stimulus.



Text-fig. 3. Response of sphincter preparation to various starfish. Contact with the crown of tentacles for 10–15 sec. produces contraction only in the case of *Dermasterias*, although the tentacles adhere strongly to *Henricia*. Rubbing the tentacles with a glass rod gives no response.



Text-fig. 4. Response of a sphincter preparation (crown of tentacles intact) to contact with whole *Dermasterias* and to extract.

The effectiveness of extract appears to fall off rapidly with dilution. In one experiment, 1 ml. of extract which had been diluted 1 in 10 had no effect. Control tests using only 0·5 ml. of undiluted extract (prepared as above) provoked sphincter contractions both before and after the trial of ineffective diluted extract. Such results indicate a sharp concentration threshold. When whole *Dermasterias* is used instead of extract, a high local concentration of the chemical substance secreted at the surface of the starfish presumably obtains on contact with the tentacles, and it is possible that the mechanical stimulus also provided by contact may be irrelevant. It is likely that these characteristics of the response, and its relatively short latency, reflect the properties of receptor elements present at least in the tentacles.

Properties of the sphincter muscle

A few observations were made on the response of sphincter preparations to electrical stimulation. Shocks were given with a Labtronics thyratron-discharge stimulator, using silver/silver chloride electrodes, and contractions were recorded with a light spring lever. The effects of electrical stimulation have been more extensively examined by Hoyle (1960).

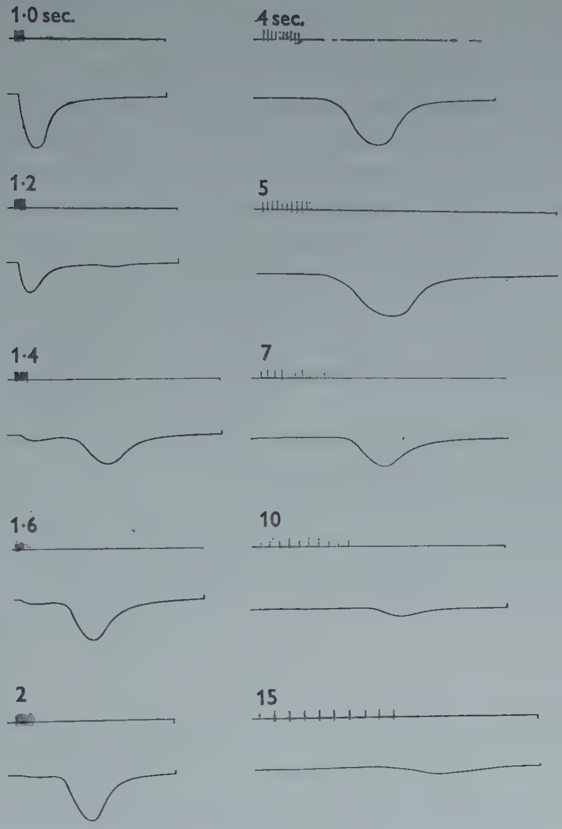
Preparations of the sphincter muscle show both quick and slow contractions. As in *Metridium* (Batham & Pantin, 1954; Ross, 1960) and *Calliactis* (Pantin, 1935*b*; Ross, 1957), quick contractions, showing facilitation, are obtained at frequencies above 1 in 3 sec., whereas slow contractions only are obtained at frequencies below this. A slow contraction may, however, follow a quick one, and at shock intervals of 1 or 2 sec. both are obtained. These effects are shown in Text-fig. 5. As in *Metridium* and *Calliactis*, quick and slow contractions appear to have the same electrical threshold (see Text-fig. 7A).

A sphincter preparation which has been stimulated for some hours may show spontaneous slow contractions. In addition, quick contractions soon begin to show a slow component as delayed relaxation. The example shown in Text-fig. 6C recalls similar observations by Batham & Pantin (1954) and Ross (1957) for *Calliactis*, by Batham & Pantin (1954) for *Metridium*, and by Horridge (1958) for *Cerianthus*. The relation between quick and slow types of contraction in such muscles, where only one type of muscle fibre is present (Grimstone, Horne, Pantin & Robson, 1958), is not yet clear.

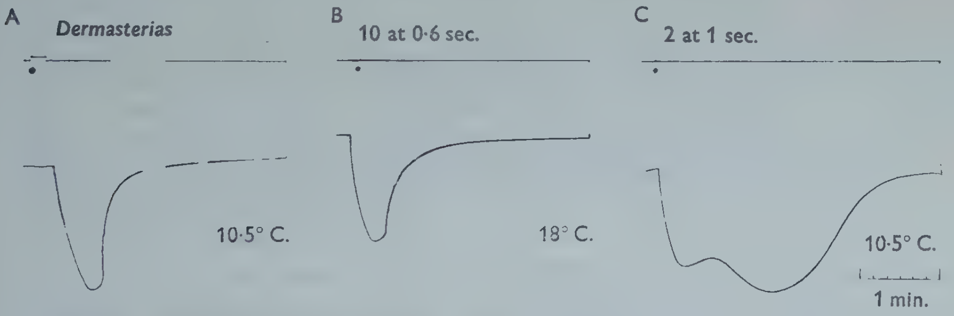
Nature of the sphincter response

Records of sphincter contractions caused by whole *Dermasterias* or by extract resemble quick responses to electrical stimulation, with the difference that relaxation is invariably rapid, whereas this is but rarely the case after electrical excitation. Text-fig. 6 illustrates two comparable contractions, and also a well-marked compound response. The characteristically rapid contraction and relaxation caused by *Dermasterias* must somehow be related to the pattern of innervation of the sphincter. The histology of this region is difficult, however, and relevant details are not yet available for any actinian. It may be noted that however carefully a mesogloeal sphincter muscle of the kind present in *Stomphia* or *Calliactis* is dissected out, some circular muscle is included in the preparation.

Dermasterias extract, whether concentrated and producing a contraction, or diluted 1 in 10 and failing to do so, appears to have little effect on the electrical threshold.

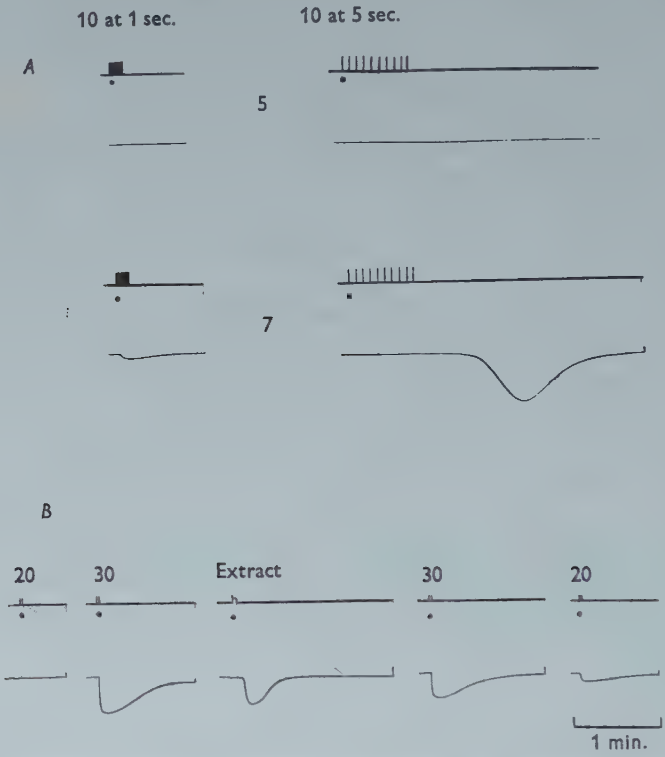


Text-fig. 5. Quick and slow contractions are obtained from a sphincter preparation according to the frequency of electrical stimulation. In this experiment each stimulus consisted of 10 shocks separated by intervals of 1–15 sec. (voltage about twice threshold value).



Text-fig. 6. Quick responses of a sphincter preparation to (A) *Dermasterias* and (B) 10 shocks at 0·6 sec. intervals, both followed by rapid relaxation. (C) A compound response to 2 shocks at 1 sec. interval, with relaxation delayed by further slow contraction.

Text-fig. 7B shows a test of the quick response to two shocks 1 sec. apart; the small change in threshold would not be unusual in the absence of extract. The unchanged neuromuscular reaction of the sphincter in the presence of extract is further evidence that the specific response to *Dermasterias* may be attributed to receptor elements. The fact that immediately after a response to *Dermasterias* the anemone is inert to renewed stimulation with starfish or extract, suggests further that sensory adaptation to the starfish substance may occur. It should be pointed out, however, that *Anemonia* has been shown to adapt slowly to the chemical stimuli of food substances (Pantin & Pantin, 1943). Most vertebrate chemoreceptors are considered to show slow sensory adaptation, although in the mammalian tongue the initial frequency of impulses from single fibre preparations of chemoreceptors falls off rapidly to a maintained lower level (Pfaffman, 1941; Beidler, 1953). Conclusions cannot therefore be drawn with regard to *Stomphia* without further experiments.



Text-fig. 7. Electrical threshold of sphincter preparations. (A) Identical thresholds of quick and slow contractions, tested with 10 shocks at intervals of 1 and 5 sec.; 5 and 7 are arbitrary voltage values. (B) *Dermasterias* extract has little effect on electrical threshold of the quick response. There is an interval of 5 min. between tests (2 shocks at 1 sec. interval).

Circumstantial evidence for the mode of action of *Dermasterias* chemical is provided by the response of independent effectors. The adhesion of tentacles, indicating the discharge of nematocysts, seems to parallel the sensory response. In these experiments, the tentacles did not adhere to glass rods, to ineffective starfish (except *Henricia*, which may occasionally cause swimming (Ward, 1958)), or to *Dermasterias* during the time after a response during which renewed stimulation had no effect. This suggests that the *Dermasterias* chemical may have a general effect at the tentacle surface rather than a particular one on receptors. Existing knowledge of how chemical and mechanical stimuli are concerned in the feeding response (Pantin & Pantin, 1943) and the discharge of nematocysts in *Anemonia* (Pantin, 1942) does not contradict this idea.

EXPANSION

After stimulation by *Dermasterias* the anemone expands much beyond its normal resting size. As the sphincter relaxes, a wave of contraction passes towards the foot, and the column elongates rapidly. This is accompanied by complete relaxation of the crown of the anemone, which becomes expanded to the point of translucence. This condition is not produced by any other 'natural' stimuli, such as feeding or the absence of light.

Elongation of the column is due to contraction of the circular muscle, whose tone is maintained throughout swimming (see p. 357). Circular contraction is propagated from the sphincter region after the initial response: this may be demonstrated in a mid-column strip of circular muscle, which contracts slowly when a small tag of sphincter and tentacles at one end is stimulated.

The expansion of the disk, sphincter and tentacles seems to be due to hydrostatic pressure maintained by the contracted circular muscle of the column, for isolated sphincter preparations do not relax beyond the initial baseline after stimulation with *Dermasterias*, as they might if some kind of neuromuscular inhibition occurred. Once a responding anemone begins to elongate, water rapidly enters the coelenteron through the mouth; one anemone, for example, took in water equal to one-sixth of its previous volume.

DETACHMENT

This phase is variable, as an anemone may show swimming movements without detaching from the substratum. More usually it will appear to detach itself with a vigorous upward jerk. In some cases, the reaction may spread from one edge of the pedal disk, or the foot may contract evenly in diameter as it lifts away from the surface. Sometimes the process is incomplete.

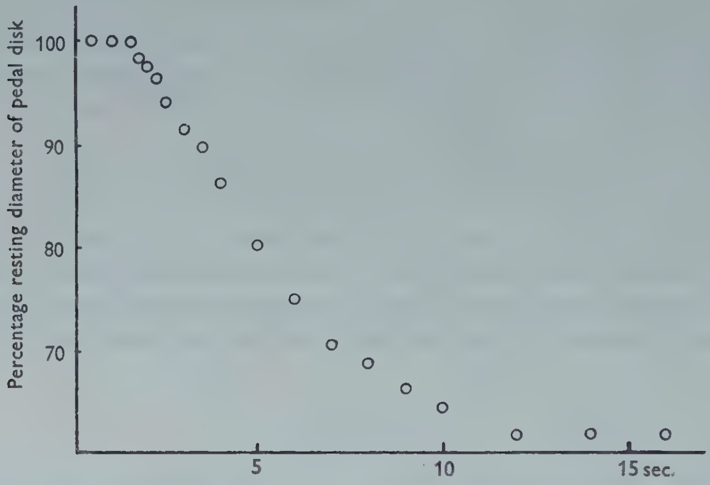
Observation of anemones detaching from a glass surface suggest that this activity has three components:

(*a*) The 'jerk' is due to contraction of the parieto-basilar muscles, which lift the pedal disk from the surface. The centre of the foot, which has no mesenteries attached (p. 345), becomes convex owing to the increased coelenteric pressure and thrusts the anemone upwards. If the parieto-basal contraction spreads more slowly from one side, forming a concentric groove (see p. 344), detachment proceeds gradually.

(*b*) At the same time, the pedal disk decreases in diameter. Although this is partly due to the wave of circular contraction which reaches it from the column, the rate at which this process occurs suggests that the basilar muscles may contract as well (Text-fig. 8; p. 346).

(*c*) Slow motion films show that a wave of opacity passes very rapidly across the pedal disk before the anemone is released from the surface. This might be a local contraction of the epithelium (see below).

These features can be related to the structure of the pedal disk. As shown in Pl. 2*c*, fibres of the parieto-basilar muscles pass right through the mesogloea of the disk. This condition is not usual in actinians and must increase the effectiveness with which the parieto-basilars release the anemone from a surface. In addition, the ectodermal epithelium contains numerous cells which give the impression of tough vertical strands. As seen in Pl. 2*d*, the proximal parts of many of the cells stain strongly with acid fuchsin. The basal parts of the cells end bluntly in the mesogloea, and the epithelial surface is covered by a cuticle. Batham & Pantin (1951) give a similar picture for *Metridium*. If the array of vertical strands shown in Pl. 2*d* is contractile, it may be responsible for the local wave of epithelial detachment noted above. There could, moreover, be functional co-ordination with the parieto-basilar system, since the fibres of these muscles appear to make contact with the epithelium.



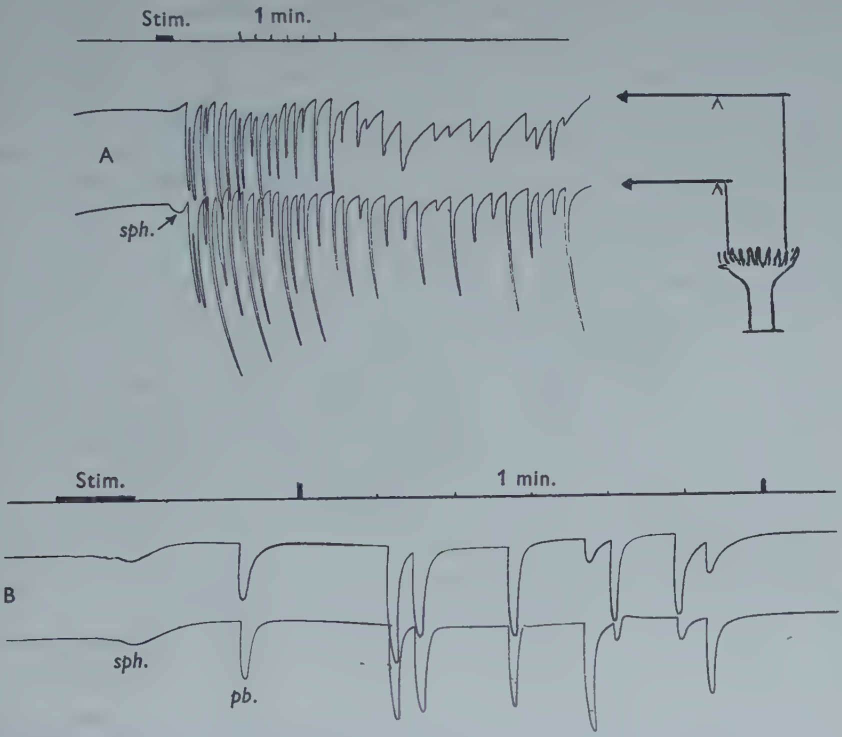
Text-fig. 8. Contraction in diameter of the pedal disk before detachment, measured from a cinefilm record, possibly indicating contraction of the basilar muscles (compare Text-fig. 5).

SWIMMING

Orientation

Whether or not detachment occurs, actual swimming movements are preceded by elongation of the column. They may be recorded from an anemone held in a fixed position. Text-fig. 9 gives some idea of swimming activity shown in these conditions. The anemone is fastened to a wax dish a day or two before, by means of fine pins; the dish is then held stationary in a large vessel of sea water by means of vaseline. Two threads, previously sewn into the edge of the disk at opposite points, are attached to light spring levers and record movements of the column.

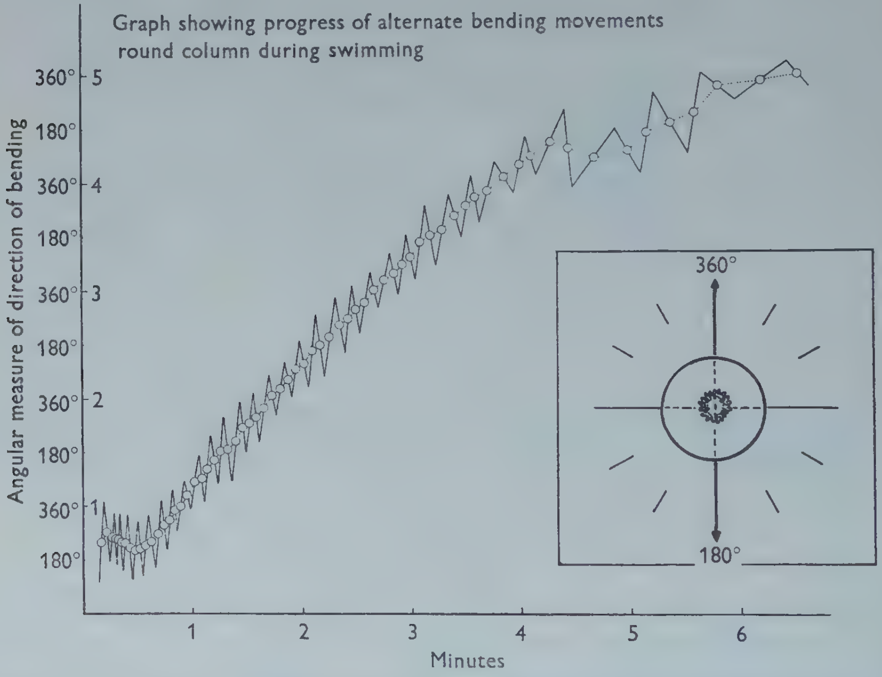
Text-fig. 9A is part of a slow kymograph record of a swimming response after a *Dermasterias* was placed in contact with the tentacles of the anemone for about 10 sec. (Stim). The initial retraction shows as a small hump (*sph.*) at the beginning of the lower tracing. Re-expansion and elongation of the anemone are indicated by the rising baseline of the upper tracing in particular, and both levers then record the ensuing series of bending movements of the column. Swimming movements may continue for several minutes, during which, as may be seen in Text-fig. 9A, their frequency falls away. Discrete contractions of the parieto-basilar muscles make the column bend first to one side, then to another, in rapid succession. Although Text-fig. 9A suggests that the contractions vary in amplitude, the apparently irregular height of successive contractions reflects instead their orientation in relation to the position of recording threads on the disk. A parieto-basilar contraction on the same radius as a recording thread gives a maximal deflexion of that lever, for example, but will hardly affect the tracing of the other lever; this is seen in the last contraction of the record in Text-fig. 9A. On the other hand, a bending movement directed midway between the attachments of the recording threads will deflect both levers equally, as in the first two parieto-basilar contractions of this record, and so on. Text-fig. 9A therefore shows that bending movements occur at a variety of points on the circumference in relation to the position of the attached threads.



Text-fig. 9. Record of a swimming anemone, with threads attached to opposite points of the disk. Stimulus mark indicates *Dermasterias* in contact with the tentacles. (A) An almost complete sequence. Note initial retraction and sphincter response (*sph.*), followed by parieto-basilar contractions which progressively decrease in frequency. Further explanation in text. (B) A faster recording of the early part of another swimming response. After the initial retraction and sphincter response (*sph.*) the rising baseline indicates elongation and re-expansion of the anemone. The first parieto-basilar bending movement occurs shortly afterwards (*pb.*), and some of the following contractions are paired. Further explanation is given in the text.

A faster kymograph record of the swimming response, such as that shown in Text-fig. 9B, shows that while the frequency of parieto-basilar contractions is irregular, a short interval between two bends (sometimes less than a second) is often followed by a longer one. This gives the impression of paired contractions followed by a pause. There are three examples of this in Text-fig. 9B. Two such contractions are in general at diametrically opposite points of the disk, whereas it may be said that two successive bends occurring less than 180° apart are not usually paired in time. The record in Text-fig. 9B may be interpreted as follows:

The tentacles of the anemone were stimulated by contact with the arm of a *Dermasterias* (Stim). After about 8 sec. the initial sphincter closure and retraction reflex ensued (*sph.*), followed by re-expansion and elongation of the anemone (note change in baseline level). The first parieto-basilar bending movement (*pb.*) was towards a point roughly equidistant from the two recording threads. After a pause, there were two paired contractions, and another single one. The last four contractions of the record fall into two pairs, and the differences between the upper and lower tracings indicate the orientation towards opposite radii of the members of a pair.



Text-fig. 10. Orientated activity graph showing the progress of alternate bending movements round the column during a swimming response. The inset shows an anemone fixed in a stationary position above a blank stencil marked at 60° intervals. During swimming the directions of bending are marked on the stencil, and the contractions timed on a drum. Each peak in the graph plotted from these records represents a parieto-basilar contraction, and the zig-zag line is a chronological record of the sites of contraction at the circumference, relative to the directive axis of the anemone. Open circles are the mid-points of lines joining successive peaks. For further explanation see text.

The orientation sequence of the parieto-basilar contractions has been examined further. If an anemone pinned to wax is stimulated (with *Dermasterias*) to swim, the directions of bending movements can be marked on a piece of paper beneath the disk with a recording error of not more than 5°. It was found convenient to use stencilled diagrams marked with points of the compass at 60° intervals. As indicated by the inset diagram of Text-fig. 10, the anemone is centred on one of these with the directive axis as a reference line. For a complete record, the sequence of swimming movements is timed on a kymograph with a tapping key, while the direction of bending is marked on the blank stencil. From these data, the orientation of successive contractions can be plotted against time, as shown in Text-fig. 10. All the anemones were placed with their directive axes along the 0–180° axis of the stencilled compass grid. The direction of bending, i.e. the radius on which any parieto-basilar contraction takes place, is then measured as an angle in relation to this axis. In the graph shown in Text-fig. 10 each sharp peak indicates a point on the circumference of the anemone at which a parieto-basilar contraction took place, and the zig-zag line joining these points represents their chronological sequence. It will be seen that the angle between successive points (representing the sites of consecutive parieto-basilar contractions) is of the order of 180°; the beginning of the graph shows this fairly well. With the conventions used here, variation in the main part of the record ranges from about 90° to 270°.

In Text-fig. 10 the mid-points of lines joining successive peaks are shown by open circles. If the swimming anemone, bending first to one side and then to the other, maintained these parieto-basilar contractions at exactly the same opposite radii throughout, the open circles would form a horizontal line. As in fact most of the graph presents a steady slope, it can be seen that the sequence of approximately opposite contraction radii itself progresses slowly round the anemone at a speed rather greater than one revolution per minute. The time scale of this rotation is of the same order in all the records obtained. In Text-fig. 10 the sequence performs a total of almost five revolutions. The swimming movements continue for 6 min., during which (as already seen in the records of Text-fig. 9A), their frequency declines.

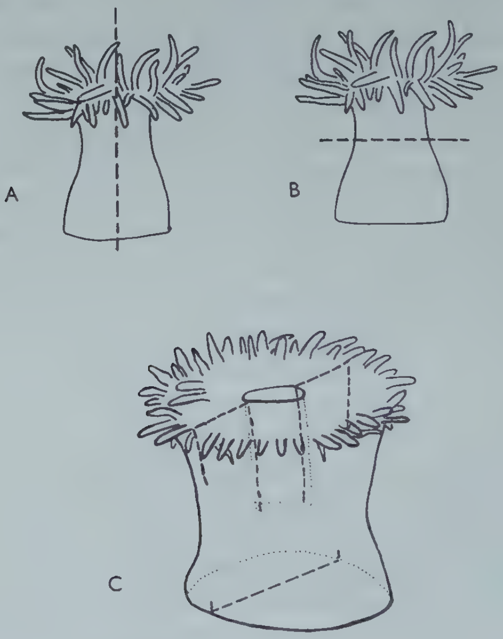
The significance of this slow phenomenon is not clear. The slow migration of the site of parieto-basilar contractions may be established only after some initial oscillation, as in the graph of Text-fig. 10. It may also reverse its direction round the anemone at any stage during swimming. The precise speed and direction of rotation seem, moreover, to be independent both of the interval between contractions, and of their actual orientations. Whatever basic excitatory process in the neuromuscular system maintains the swimming contractions (see p. 358), it is difficult to attribute this slow shift in the orientation of parieto-basilar activity to a distinct, equally specific process. If, for example, steady 'rotation' represented by the slope of the graph in Text-fig. 10 were due to a slowly conducted wave of contraction progressing round the circular muscle of the column, some initial oscillation might be expected, but not the reversals from the clockwise to anticlockwise, or vice versa, that occur in several swimming records. The phenomenon is too slow and too variable to be explained as specific activity within the nervous system or in the muscular system. Despite the well-defined slope of graphs such as that shown in Text-fig. 10, it does not seem likely that any special excitatory process responsible for the appearance of rotation in the bending sequence exists. Some kind of asymmetry in the main excitatory process may provide an explanation.

It may also be noted that graphs of the kind shown in Text-fig. 10 cannot be interpreted as a repetitive pattern associated with particular mesenteries. The six primary anatomical radii of the anemone have no special significance in this context, although it may be recalled that there are, in fact, usually eighteen pairs of perfect mesenteries (p. 345), whose parieto-basilars are those mainly responsible for the swimming movements.

In a vigorous response the column of the anemone often performs a whirling movement about its own axis before the usual abrupt bending movements begin. This whirling is recorded by Yentsch & Pierce (1955), and by Sund (1958), and represents the serial contraction of parieto-basilar muscles round the circumference of the anemone. This activity, if rapid, is the one which initially propels the animal into some semblance of 'swimming'; for the staccato contractions described above do not generally raise the anemone in the water to any extent.

Transmission of excitation

It has been observed by Wilson that both halves of a horizontally transected anemone may show the components of a swimming response to *Dermasterias*. This has been broadly confirmed, although preparations are no less variable than whole specimens. Vertical halves can also give a full response. One vertical half, and a horizontal half which included the lower pharynx and pedal disk, both continued to give proportionately accurate swimming responses for more than 5 weeks, by which time some regenerative growth had occurred. The latter specimen also demonstrated that there must be receptors sensitive to *Dermasterias* elsewhere than on the disk and tentacles (see p. 348 and Text-fig. 11 A, B).



Text-fig. 11. Diagrammatic summary of cutting experiments.

A variety of cutting experiments was carried out to determine which parts of the animal could propagate the sequence of parieto-basilar excitation. It was found that bending movements would still occur at opposite radii when these were joined only by mid-column tissue; as in Text-fig. 11 C, the disk, pharynx, upper column, and foot may be cut, and yet stimulation of the tentacles on one side with *Dermasterias* still leads to parieto-basilar swimming contractions which may take place at any point on the circumference. If only the foot, oral disk, or sphincter, or a combination of these, were transected, the sequence did not appear to be affected either. When preparations gave responses of sufficient duration for the plotting of activity graphs of the kind shown in Text-fig. 10, the progress of excitation appeared to be fairly normal. These experiments, although far from conclusive, suggest that excitation of the parieto-basilar muscles during swimming is maintained in the column.

It is relevant that the excitation of circular and parieto-basilar muscles appears to be closely correlated. When the tone of the circular muscle is high, and the anemone is fully extended, swimming movements are very vigorous. As the frequency of bending falls off towards the end of swimming circular tone is lost and the anemone increases in diameter, ultimately regaining its resting dimensions. As in *Metridium* (Batham & Pantin, 1954; Robson, 1957; Batham, Pantin & Robson, 1960), the linked activity of these two systems could be explained in terms of their anatomical situation, which could admit of a common system of innervation.

Only a few preparations of *Stomphia* were vitally stained with methylene blue. Bipolar nerve cells, much like those described for other anemones (Hertwig & Hertwig, 1879; Pantin, 1952), are present at least in the mesenteries (Pl. 2*f*). In the column, however, there are histological elements resembling multipolar nerve cells (Pl. 2*e*). If multipolar nerve cells are present in *Stomphia*, it is possible that they may be concerned with initiating or maintaining excitation in the parieto-basilar system during swimming.

RECOVERY

Detailed observation of recovery after swimming did not form part of the present study. The anemone comes to rest on its side and is relatively unresponsive to *Dermasterias* and other stimuli. The column becomes shorter, and as the tone of its circular muscle is relaxed, that of the disk and tentacles returns to normal. Yentsch & Pierce (1955), and Sund (1958), estimate that the anemone 're-establishes its hold on the substratum' after 1 or 2 min. The normal sessile position may be regained with corresponding speed, but in some cases the whole recovery phase lasts longer. Once the anemone has reattached, it will usually respond to stimulation with *Dermasterias* by swimming again.

At the stage when the pedal disk is about to reattach to the substratum it becomes extremely adhesive. The surface is thrown into fine corrugations and will fasten immediately to any solid object. The adhesion is such that the whole anemone may be lifted about on the tip of a mounted needle. The secretion of an adhesive substance, while probable (see layer of cuticle below the epithelium of attached specimen, Pl. 2*d*), would seem insufficient to account for this, and local contraction at least of the epithelium is probably involved as well (see p. 352). In an anemone lying on its side after swimming the edge of the pedal disk nearest to the substratum adheres to it, and contact is rapidly regained over the whole disk.

DISCUSSION

Although, as noted by Wilson (1959), the swimming response of *Stomphia* is variable, nearly all anemones which respond to *Dermasterias* display the following basic sequence:

$$\begin{tikzcd}
\text{Tentacle and sphincter response} \arrow[r, "A"] & \text{Expansion} \arrow[r, "B"] \arrow[dr] & \text{Swimming movements} \arrow[r] & \text{Recovery.} \\
 & & \text{Detachment} &
\end{tikzcd}$$

Detachment does not always occur and may be regarded as a side effect. Parieto-basilar swimming movements, on the other hand, do not take place unless the column has elongated; excitation at B therefore depends on A. This suggests that the parieto-basilars and circular muscle of the column are functionally co-ordinated, their excitation being parallel and interdependent. In *Metridium*, an anemone in which the activity of the column is very slow, the parietal and circular muscles, although co-ordinated, by contrast act reciprocally (Batham & Pantin, 1954). In *Stomphia*, the more elongated the column, the more vigorous the swimming activity which ensues. Excitation may persist for several minutes and seems to be maintained in a functionally distinct part of the nervous system situated in the column.

The 'column system' is activated by an initial response of the tentacles, sphincter, and possibly retractor muscles to *Dermasterias*. All observations so far suggest that whereas the rapid sphincter-retractor system can excite or inhibit the column, the converse does not hold. A swimming anemone, for example, will retract if given a sharp prod, and the column system is inhibited until the sphincter and retractors relax, when swimming is usually resumed. A one-way relation between the sphincter-retractor and column systems may possibly occur in *Metridium*, since events in the rapid through-conduction system may either excite or modify the activity of the column, but not vice versa (Batham & Pantin, 1954).

Although the swimming movements of the column are the most striking part of the whole response, it is not yet clear how they are propagated. Assuming that excitation is maintained in the column, there are two possibilities. Either each bending movement in the series depends on excitation set up by the preceding one after the manner of a chain reflex, or it is fired off by one or more pace-makers. There is little evidence for the first possibility except in the particularly short interval between two contractions when they are at diametrically opposite radii (p. 353). Here it seems as though the second contraction of a pair may be a direct result of the first. The simple explanation which would be provided by a sensory reflex is not very satisfactory. It could be supposed, for example, that every parieto-basilar contraction causing the anemone to bend excited the sense cells at the opposite radius of the column mechanically, by stretching. Sense cells in the column are endodermal, and they are aggregated along each side of the mesenteries at their junctions with the column (Pantin, 1952; Batham *et al.* 1960). It has been shown in *Calliactis* that they respond particularly to stretch and to other mechanical deformation tangential to the surface of the column. But existing evidence does not support the idea of this kind of 'stretch reflex' in *Stomphia*. Paired parieto-basilar contractions have been observed in experimentally incised anemones which were so little expanded, owing to the loss of coelenteric pressure, that no part of the column could possibly have been stretched.

The possibility remains that every parieto-basilar contraction stimulates the sense cells along its own radius. This excitation would in turn have to produce a contraction on the other side of the anemone. If excitation were conducted in both directions round the circumference from the first site of contraction, a symmetrical position of the second parieto-basilar contraction would follow. On the other hand, the third contraction very rarely occurs at the same radius as the first, and it is preceded by a longer interval than that between the first two contractions. Even allowing for refractoriness, inhibition, or fatigue in some part of the neuromuscular system at the first radius of contraction, no simple theory of sensory reflexes can fully explain the contraction sequence of the parieto-basilar muscles.

To whatever extent sensory reflexes may be involved in the pattern of swimming movements, the nervous system plays an important part in this activity. It does not seem likely that chain reflexes of nervous, any more than of sensory origin, could alone account for a series of parieto-basilar contractions lasting for several minutes, and some kind of pacemaker system most probably exists. This might be localized in one region, or else consist of several active sites, which would not necessarily occupy fixed positions. It is possible, as suggested above (p. 357), that one or several pacemakers are situated in the column. Whether or not this can be confirmed, much more information about the neuromuscular system of *Stomphia* is required before a satisfactory explanation for the type of record shown in Text-fig. 10 can be put forward (see Hoyle, 1960).

There are few records of swimming in other actinians. Planktonic forms (e.g. *Minyas*) float passively, and *Aiptaisia* (Portmann, 1926) can creep slowly by performing burrowing movements when in a horizontal position. The only published description of rhythmical activity as rapid as in *Stomphia* seems to be Verrill's note (1928) on *Nectothela lilae.** This is a small Hawaian anemone related to *Anemonia*, which possesses long and extremely numerous tentacles. It can swim actively, apparently by rhythmical contractions of the disk, during which time the column is markedly shortened. Here a temporary pacemaker is almost certainly present. Carlgren referred *Nectothela* to *Boloceroides mcmurrichi* and found a similar species in the Red Sea (1899, 1927, 1949), and it is also recorded from Japan (Uchida, 1938). *Gonactinia prolifera* can also swim, apparently in the same way (Stephenson, 1935).

Although there is little information about the distribution of the nervous system and receptors in *Stomphia*, actinian nerve cells usually run between muscle fibres and the epithelium, and thus in general follow the plan of the muscles (Batham *et al.* 1960). Sections of *Stomphia* show that neurites in this situation could easily link up the responding muscle systems without passing into the mesogloea, and thus transmit excitation between them. Future histological studies may be expected to reveal nervous connexions between the tentacles and sphincter; between the sphincter and the retractors on the one hand, and the circular/parieto-basilar system of the column on the other; and between this last system and the pedal disk. In the absence of more direct information, inspection shows that anatomical junctions between the different muscle systems are sufficient to account for most of the pathways of excitation which are indicated by the animal's swimming behaviour.

The effectiveness of actinian muscle systems depends upon the hydrostatic skeleton provided by sea water in the coelenteron (Batham & Pantin, 1950*a*; Chapman, 1958). In this respect, *Stomphia* may be regarded as an almost watertight cylinder, for the mouth is the only opening (p. 345). The mesenteries partition the coelenteron so efficiently that vertical halves of a cut anemone still maintain approximately normal tone instead of collapsing. Coelenteric pressure has not been measured in *Stomphia*, but in *Metridium* it averages 2–3 mm. H_2O (Batham & Pantin, 1950*a*). Bending movements of the column during swimming depend on the turgor of the crown region, which is maintained by circular tone of the column. The parieto-basilar muscles, bending the crown towards corresponding radii, act through mesogloeal 'tendons' in the upper regions of the mesenteries (p. 346). In anemones incised for experimental purposes, the crown may fail to expand and parieto-basilar contractions then simply produce grooves in the disk.

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* Mr R. Josephson kindly drew my attention to *Nectothela*.

The initial closure of the anemone forces out coelenteric fluid through the mouth. In the rapid expansion which follows, water is taken in again so quickly that ciliary action of the siphonoglyphs would hardly seem adequate. In *Metridium*, Batham & Pantin (1950*a*) found that anemones recovering from extreme contraction could show negative coelenteric pressures. Their suggestion that suction might be exerted by peristalsis would also apply to *Stomphia*. In one specimen, the over-all specific gravity could decrease from 1·08 to 1·06, implying a significant increase in buoyancy during swimming. In natural conditions, a swimming anemone would be carried much further by currents than its own random movements might suggest. Sund's underwater observations bear this out, as one specimen travelled 1½ m. while he watched it.

The response of *Stomphia* to certain starfish has parallels among some of the associations between other marine animals. Several commensals, for example, show behaviour preferences for host-specific chemical substances (Davenport, 1955). Perhaps the best-known active response to starfish is the clapping reaction of the scallop *Pecten*, which can hardly be interpreted as other than an escape mechanism. Lecomte (1953) found that both mechanical and chemical stimuli were needed, and that the latent period of the response varied according to the species of starfish used. Bullock (1953) has reported the flight reaction of the gastropod *Nassarius* induced by starfish, and Clark (1958) has shown that herbivorous littoral gastropods will move rapidly away in the presence of certain carnivorous ones.

The action of reduced glutathione on *Hydra* (Loomis, 1955) provides an example of prolonged activity induced by a known chemical substance. In suitable concentration it evokes a complete feeding reaction. The gastrozooids of the siphonophore *Physalia* react in the same way (Lenhoff & Schneiderman, 1959), but the substance is without effect on several species of sea-anemones, including *Stomphia* (Passano, 1957). The responses of anemones to chemical stimuli may be seen, however, in feeding (Pantin & Pantin, 1943) and spawning behaviour (Nyholm, 1949). In general, dissolved chemical substances affect at least the slow, phasic activity of the column (Batham & Pantin, 1950*c*), and often that of the crown and foot as well.

The swimming movements of *Stomphia* differ in their rapidity, however, from the much slower activities of anatomically comparable regions in other sea-anemones. In *Metridium*, walking, for example, is a complex activity involving the pedal disk and lower column (Parker, 1919; Pantin, 1952). It is extremely slow compared to the rapid reactions of the pedal disk in *Stomphia*, but both can take place in transected lower halves of anemones, and the neuromuscular co-ordinating mechanisms may be similar. Some approach to the localized parieto-basilar response of *Stomphia* is found in the Brazilian anemone (?*Bunodactis*) studied by Pantin & Vianna Dias (1952). It responds to gentle prodding by rapid flexion of the column. This is much faster than the slow parietal shortening which is produced in most anemones by local illumination and other stimuli. Time-lapse films show that when much expanded in the presence of food, *Metridium* may also perform slow bending movements, in which the animal sways, or flexes at the base of the column. Batham & Pantin (1950*a*) interpreted the latter as a tendency for the anemone to overbalance when its coelenteric pressure is very low, and these sweeping movements do not seem to have much in common with parieto-basilar contractions.

It seems possible to derive various components of *Stomphia*'s swimming response from ordinary patterns of behaviour in other actinians, but this throws no light on how such a response could have evolved, especially as its biological significance is still obscure. Specialized anatomical features in *Stomphia* reflect its ability to swim, and it is possible from Carlgren's record (1921) of the allied deep-water *Actinostola spetsbergensis* that other anemones in this family may also swim.

Of the two starfish known to cause swimming in *Stomphia*, *Dermasterias imbricata* is the only species of its genus, and is strictly indigenous to the Pacific coast of North America (Fisher, 1911). *Hippasteria spinosa* has a similar distribution, but other species occur further north, and *H. phrygiana* ranges from Scandinavia to North America across the Atlantic. It is thus possible to correlate the distribution of *Hippasteria* with that of *Stomphia* to a certain extent (see p. 343). *S. coccinea* dredged off the north coast of Denmark gives a strong swimming reaction to specimens of *H. phrygiana* which occur occasionally in that area (Dr Gunnar Thorson, personal communication). According to Mortensen (1927) *H. phrygiana* occurs at depths between 20 and 800 m., and feeds on echinoderms, mussels and worms. If *Hippasteria* fed on *Modiolus*, which seems to be a fairly common substrate for *Stomphia*, it is just possible that the anemone might swim as an escape reaction. On the other hand, if live specimens of *Stomphia* can be obtained outside the range of *Dermasterias* and *Hippasterias*, and different animals are found able to excite a swimming reaction, the problem will present yet another facet of peculiar interest.

SUMMARY

1. The swimming behaviour of the anemone *Stomphia coccinea*, previously known to be a specific response to contact with two species of starfish, is briefly described.

2. The sphincter, isolated or intact, gives a reflex quick contraction when the tentacles are stimulated with whole *Dermasterias imbricata* or with extract, but not when other starfish are used. Sphincter preparations stimulated electrically give a quick facilitated contraction at frequencies above 1 in 3 sec., and slow contractions at frequencies below 1 in 2 sec. Quick and slow contractions appear to have the same threshold, which is unaffected by the presence of starfish extract. Possible attributes of the chemosensory system are discussed.

3. Elongation of the column is an essential preliminary to swimming, whereas detachment from the substratum, due mainly to contraction of the parieto-basilar muscles, does not always occur. Cutting experiments, and orientated recording of the swimming movements which ensue, suggest that excitation is maintained locally in the column, and that the parieto-basilar and circular muscles function synergically. Multipolar nerve cells in the column may be concerned with this activity.

4. Various special features of the anatomy of *Stomphia* may be correlated with its ability to swim.

5. Although the origin and adaptive value of the swimming response are still obscure, it is clear that it must be of distinct biological advantage to the species.

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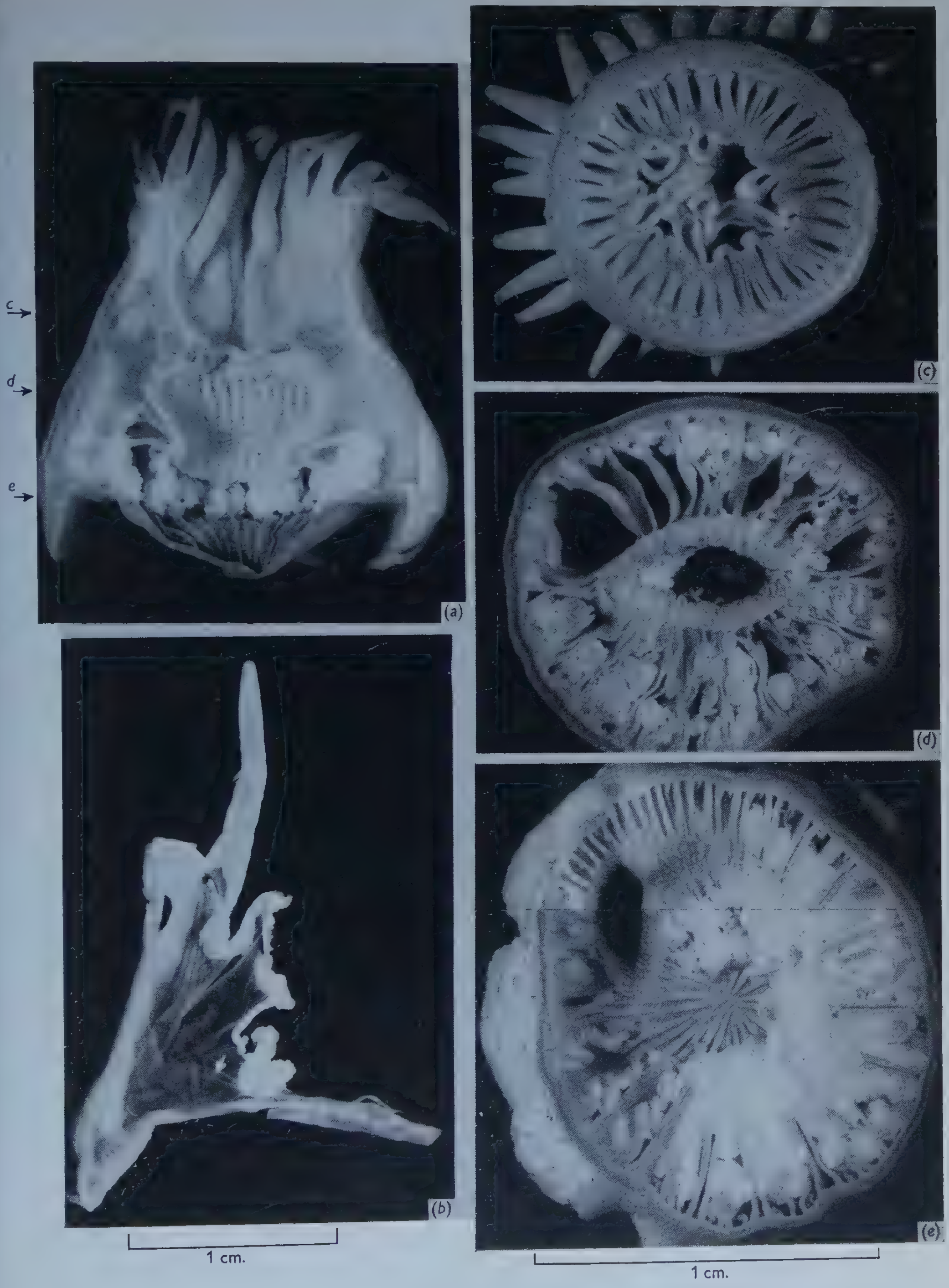

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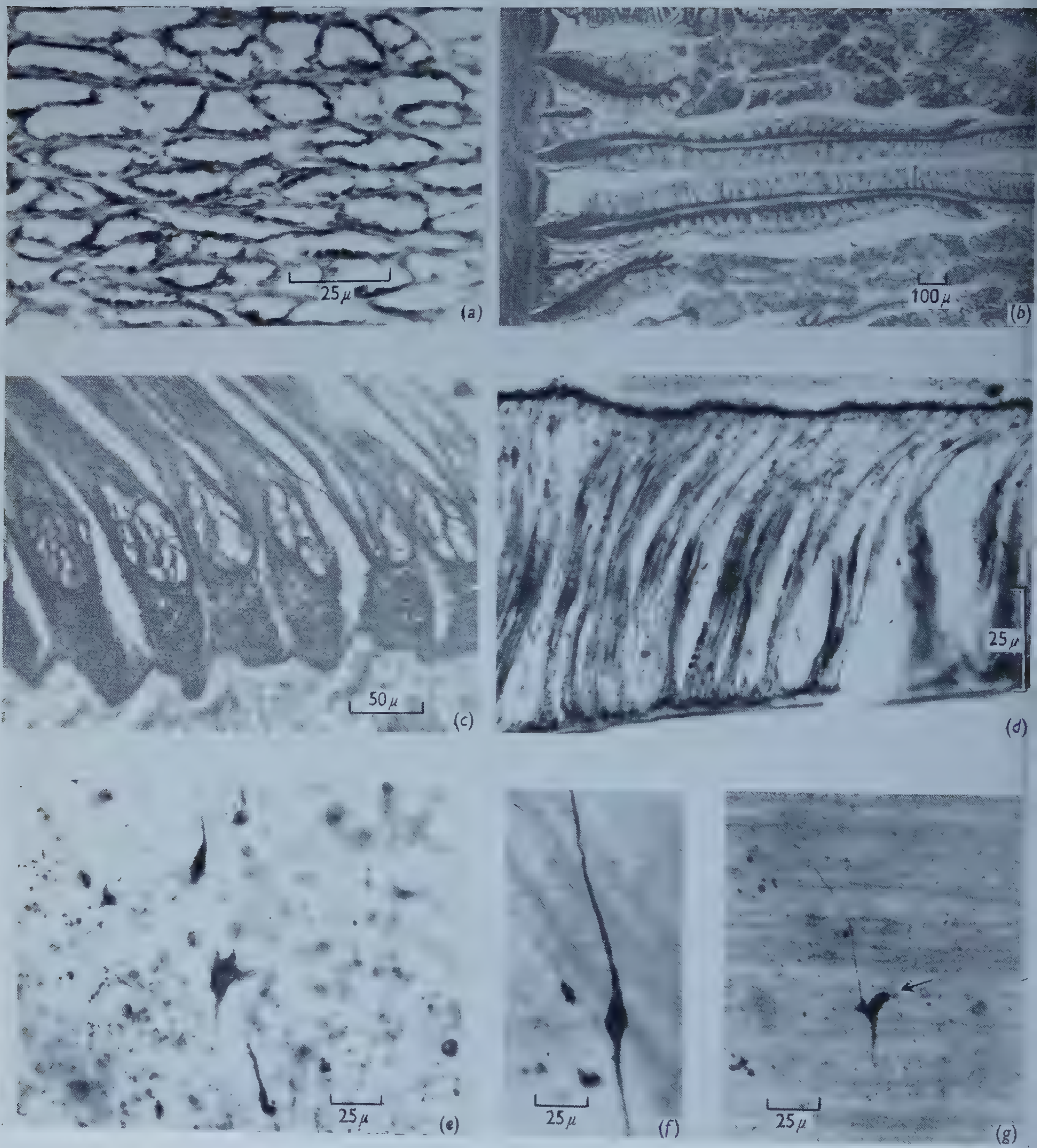

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EXPLANATION OF PLATES

PLATE 1

Anaesthetized specimens fixed in picroformol.

(*a*) Vertical half of specimen with crown only partly relaxed.

(*b*) A perfect mesentery, with its attachment to pharynx, disk, body wall and foot. Note especially the vertical folds of the retractor muscle (endocoelic), and the triangular parietobasilar muscle (exocoelic) whose border is shown by the arrow. Part of the opaque region of the mesentery which continues upwards from this muscle (top left) is a thickened strip of mesogloea (see Text-fig. 2).

(*c*), (*d*), (*e*) Surface views of thick slices taken at approximately the levels shown by the arrows in (*a*).

PLATE 2

(*a*) From a transverse section of sphincter (Susa–Mallory), showing muscle fibres lining cylindrical spaces in the mesogloea. The material is poorly preserved.

(*b*) Transverse section showing the junction of two perfect mesenteries with the column (to the left). Note endocoelic retractor muscles, and the parieto-basilars, developed as a fold on the outer surface of each mesentery. The apparent split in the mesogloea of each septum indicates the position of exocoelic radial muscle fibres running in a transverse direction (Susa–Masson).

(*c*) Vertical section of the foot along a radius. The ectodermal epithelium is just seen at the bottom of the picture. Between the oval cross-section of bundles of circular muscle fibres embedded in the mesogloea oblique spaces run towards the epithelium. The fibres lining these spaces are those of the parieto-basilar muscle, which thus make contact with the surface of the foot (Susa–Mallory).

(*d*) Highly magnified portion of epithelium from the foot (mesogloea at top of photograph). Note numerous vertical strands which have stained with acid fuchsin, their blunt terminations in the mesogloea, and the cuticle covering the surface (Susa–Mallory).

(*e*), (*f*), (*g*) From preparations fixed after vital staining with reduced methylene blue. (*e*) A multipolar nerve cell from the mid-column region of the body wall. (*f*) Central part of a bipolar nerve cell from the retractor face of a perfect mesentery. (*g*) Sense cell with two neurites, and the flagellum just visible at the arrow, from the pedal disk.

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THE HYDRODYNAMICAL PERFORMANCE OF MIGRATORY SALMON

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INTRODUCTION

It has been known for a long time that fish and aquatic animals such as porpoises apparently swim with little effort. Attempts to observe or estimate indirectly their energy requirements have frequently, but not always, led to one of two conclusions: (1) these creatures have a much smaller drag than could be achieved with similar, man-made vehicles; (2) the power output per gram of muscle (ergs/sec.-g.) is considerably larger than might reasonably be expected from physiological experiments on man, or other warm-blooded animals (Gray, 1948, 1949).

There is, therefore, considerable uncertainty at present in the available information to answer quantitatively the question: how well do fish swim? It is the purpose of this paper to examine a natural phenomenon in order to answer this question. This phenomenon is the annual spawning migration of salmon up the Columbia and Amur rivers. In these migrations the following facts can be accepted as well established. Fish weighing from 3 to 40 pounds go distances as great as 1000 miles upstream to spawn and die. It is known that the fish do not eat after leaving the sea, and the weight and chemical composition (as fat, protein and water) both of the fish and the 'delivered payload' of reproductive products, at various stages of the migration are reasonably well established. Data on the velocity of the river throughout the year are also available. Fig. 1 shows the elevation of the Columbia River system versus river mile from the mouth, the major tributaries and places where salmon were known to have regularly appeared in the past (Bryant & Parkhurst, 1950; Parkhurst, 1950*a*, *b*). Fig. 1 can be used to locate by river mile (R.M.) places mentioned in the text.

Such a migration constitutes, from an engineering standpoint, an experiment on the maximum range of an unrefuelled vehicle. From the above data one can determine, at least in principle, the orders of magnitude of the energy consumption and drag which must obtain for the fish to reach its destination.

In this paper we shall compare the performance of the fishes as measured by fuel (fat) consumption with the fuel requirement of hypothetical rigid vehicles of the same length and total surface area, traversing the same reaches of the river on the same time schedule as the salmon migration. To these vehicles we shall assign the best reasonable engineering specifications for large efficiency and low drag presently known. If the computed fuel requirements of our rigid vehicles are larger than the observed fuel available to real fish, one must suppose that the fishes do indeed have abilities superior to those of our 'best vehicle', or else seek other possible explanations.

The reason for examining the performance of migrating salmon by making this comparison with rigid vehicles is that we do not know how to calculate the propulsion or drag of non-rigid vehicles. Except for the excellent work of Sir Geoffrey Taylor (1952) on swimming snakes, there seems to be neither a theory nor appreciable experimental data for making such a calculation.

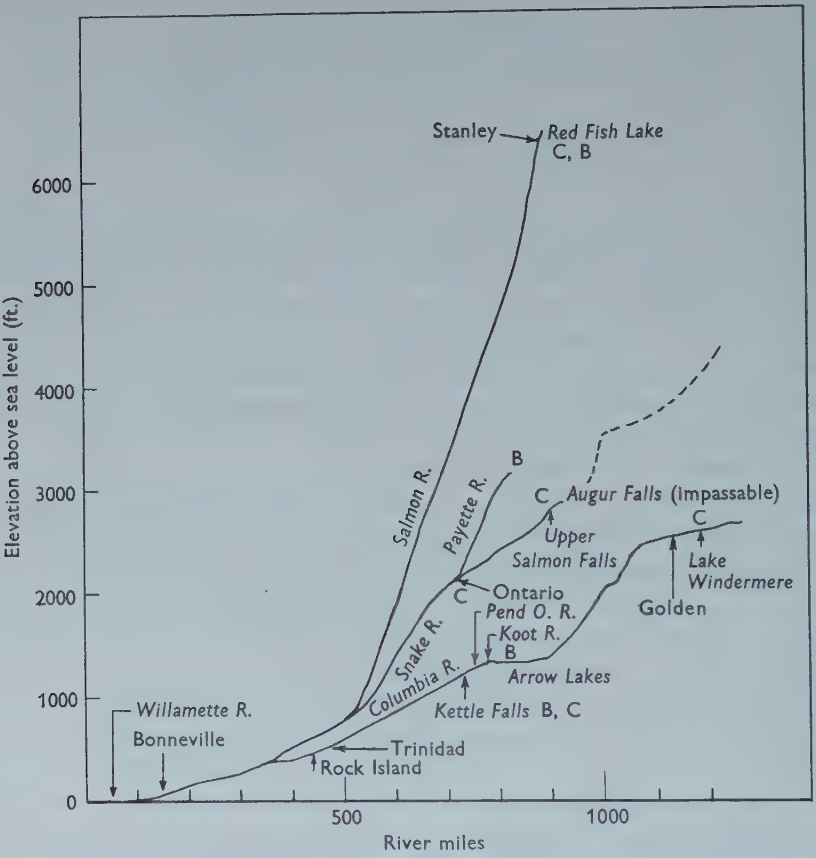

Fig. 1. Elevation above sea level *vs.* river mile for Columbia River system, with points to which chinook (C) and blueback (B) salmon are known to have migrated.

Let us express the force on our hypothetical rigid vehicle as follows: S is its surface area, which we shall take as equal to the total surface area of the corresponding fish, including both sides of the fins and tail. ρ is the water density, V_W is the velocity of the water downstream relative to the bottom of the river, V_F is the velocity of the vehicle (cf. fish) upstream relative to the bottom. C_f is the skin friction coefficient. Then it is well known that the drag force F (in dynes, c.g.s. units) on the vehicle is at least as large as the following expression (Goldstein, 1938).

$$F = (\tfrac{1}{2})\,\rho C_f S(V_F+V_W)^2. \tag{1}$$

For well-polished, streamlined objects the total drag force is in fact only slightly greater than that given by this expression, provided the 'appropriate' value of C_f is used. Equation (1) expresses the drag on a flat plate of total (wetted) area S, dragged edgewise through the water.

Two choices of C_f are possible. For a laminar boundary layer

$$C_{f(\text{lam})} = 1{\cdot}33\sqrt{R}.$$

For a turbulent boundary layer

$$C_{f(\text{turb})} = 0{\cdot}455\,(\log_{10} R)^{-2{\cdot}58}.$$

R is the Reynolds number = length of vehicle × $(V_F + V_W)$/kinematic viscosity. For the length of our vehicle we shall take the length of the corresponding fish, measured to the narrowest point of the tail.

Generally speaking, for large Reynolds numbers (greater than 10^6), $C_{f(\text{turb})}$ is appropriate, and $C_{f(\text{lam})}$ for smaller values. The precise value of R for transition from laminar to turbulent flow in the boundary layer depends on surface roughness, the degree of turbulence in the water, and the details of the shape of the specific vehicle being considered.

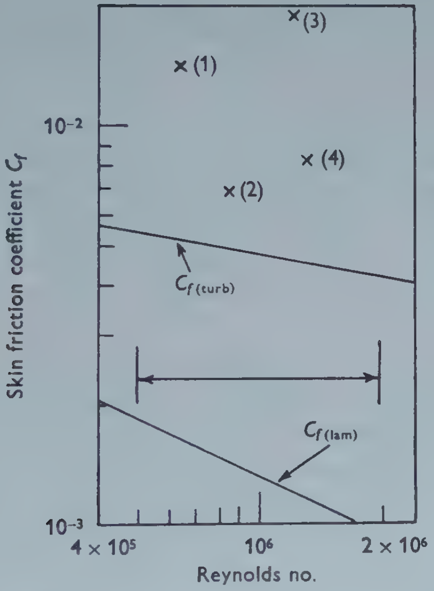

Fig. 2. Theoretical skin friction coefficients and observed total drag coefficients referred to entire surface area of dead fish and models. (1) Bowfin, from Gero (1952); (2) plaster dogfish model, without fins or tail, from Harris (1936); (3) flexible paralysed pike, and (4) rigid waxed pike from Kempe (1935). The double arrow denotes the range of Reynolds numbers occurring for salmon in this paper.

In Fig. 2 we have plotted these two values of C_f, and also some experimental values of the total drag coefficient for dead fish and fish models, referred to the total surface area. In computing the fuel consumption of our vehicles, we shall use $C_{f(\text{turb})}$, and it will be seen that this choice ascribes to our vehicles drag forces about one-half to one-third those actually observed for dead fish, but still considerably larger than the very least value using $C_{f(\text{lam})}$ that might be achieved by highly polished, carefully contoured vehicles in smooth water. We have also marked in Fig. 2 the range of Reynolds numbers for the fish considered in this study, which is also roughly the range in R for transition from a laminar to a turbulent boundary layer.

The power P (ergs/sec.) expended in moving the vehicle with a speed V_F+V_W relative to the water is at least as large as

$$P = (\tfrac{1}{2})\, C_f \rho S(V_F+V_W)^3. \tag{2}$$

The corresponding energy E in ergs (lower limit) expended by the vehicle in traversing a distance X (cm.) relative to the bottom of the river, with velocity V_F (cm./sec.) is $E = PX/V_F$, which we can write in the form

$$E = (\tfrac{1}{2})\, \rho C_f S V_W^2[(1+V_F/V_W)^3\,(V_W/V_F)]\, X. \tag{3}$$

This assumes for the moment that V_F and V_W do not vary over the interval of time X/V_F, nor explicitly with the position in the river.

In view of the way biological data is given on the chemical analysis of fish, let us convert the energy E above to a fuel requirement, expressed as grams of fat, which is the major fuel of real fish. W grams of fat will yield on combustion with oxygen WHJ ergs, where H is the heat of combustion ($H = 9{\cdot}3$ kcal./g. of fat) and J the mechanical equivalent of heat ($J = 4{\cdot}18 \times 10^{10}$ ergs/kcal.). We postulate an efficiency N_1 for converting chemical energy (the heat of combustion) to mechanical energy in the 'engine' of our vehicle. We also postulate an efficiency N_2 for converting mechanical energy of the 'engine' to directed kinetic energy in the water. N_2 corresponds to the efficiency of a propeller (analogous to the tail of the fish) in converting the rotational energy of an engine to translational energy in the wake. In practice $N_1 \leqslant 0{\cdot}3$ for human beings or a good internal combustion engine, and $N_2 \leqslant 0{\cdot}8$ for the very best propellers. We shall assign these optimum values to our vehicle, giving an overall efficiency of 24% from heat of combustion to directed kinetic energy. So we have to traverse the distance X

$$WHJN_1N_2 = (\tfrac{1}{2})\, \rho C_f S V_W^2(1+V_F/V_W)^3\,(V_W/V_F)\, X. \tag{4}$$

We can use equation (4) to give the fuel requirements terms of grams of fat per 100 g. of loaded vehicle of initial mass M, at the mouth of the river. At the same time we can take into account the fact that V_F and V_W may vary as our vehicle progresses up the river, by dividing the length of the river into reaches denoted by the subscript i (100 miles in length for the case of the Columbia) and evaluate V_{Fi}, V_{Wi} for each particular reach at the particular time of year, or schedule date for the vehicle's passage. Adding up the fuel requirement for each reach, from the mouth to spawning grounds, we have for ($100\, W/M$), the fuel requirement per 100 g. of initially loaded vehicle of mass M,

$$100\, W/M = (100/MHJ)\,(\tfrac{1}{2})\, \rho \Sigma_i (C_f/N_1N_2)\, S V_{Wi}^2(1+V_{Fi}/V_{Wi})^3\,(V_{Wi}/V_{Fi})\, X_i. \tag{5}$$

It is the quantity computed from the right-hand side of equation (5) which is to be compared with the corresponding fuel consumed by real fish, from changes in their weight and chemical composition.

We have written equation (5) in the form given in order to show the various sources of uncertainty. The factor C_f/N_1N_2 we have chosen to be as small as we may reasonably assume. The factor $V_W^2(1+V_F/V_W)^3\, V_W/V_F$ shows the most economical way in which our vehicle should be operated.

Let us suppose for the moment that in a given reach of length X_i, the water velocity V_{Wi} is fixed, and not subject to the vehicle's choice. What choice of the operating speed V_{Fi} uses the least fuel to cover the distance X_i? This is at $V_{Fi} = \tfrac{1}{2}V_{Wi}$. This neglects the small dependence of C_f on velocity via the Reynolds number. However, this minimum is a rather broad one, so that in the range $0{\cdot}3\ V_{Wi} < V_{Fi} < V_{Wi}$ the fuel consumption is within 15% of the minimum value. This gives our vehicle considerable latitude in its choice of operating speed V_{Fi}, subject only to the proviso that the reach must be covered in a time interval less than or equal to that observed for real fish. The vehicle must, in other words, stay on schedule with real fish.

If we now allow the vehicle some choice for V_{Wi} in the reach of length X_i, then in the neighbourhood ($0{\cdot}3\ V_{Wi} < V_{Fi} < V_{Wi}$) of the optimum $V_{Fi} = 0{\cdot}5\ V_{Wi}$ the fuel requirement varies approximately as the square of V_{Wi}. This means, subject to the same proviso as before, that there is considerable fuel saving to be obtained by operating the vehicle in low-velocity water, such as might be found close to the bottom, or the shoreline.

This completes the discussion on the general computation of fuel requirement for a vehicle progressing with velocity V_F against a counter flow V_W. In the next two sections we consider the determination of numerical values for V_W and V_F.

THE VELOCITY OF THE WATER

The basic equation for determining river velocities is a simple expression of continuity for the steady flow of an incompressible fluid. This states (using units in which the data is published) that the volumetric rate of discharge, D, in cu.ft./sec. equals the velocity V_W (ft./sec.) times the cross-sectional area A (ft.^{2}) at right angles to the flow. In terms of V_W

$$V_W = D/A. \tag{6}$$

Note that V_W is the average velocity over the entire cross-section.

The U.S. Geological Survey and the Canadian Water Resources Board have kindly provided the author with unpublished data giving the discharges, areas and hence average velocities at the various metering stations on the Columbia (Fig. 3).

The published record (USGS Water Supply Papers, U.S. Army Engineers Report on the Columbia, 1948) gives the discharge versus date at the metering stations, plus a table of the elevation of the river surface above sea level at the station, as a function of discharge. This is the ‘rating curve’. Daily records of this type go back as far as 1876 at The Dalles, 200 river miles from the mouth. This published record plus the previously unpublished data of Fig. 3 enables the average velocity at a metering station to be determined on any date for which the discharge was known.

Since we shall be using the discharge averaged in a number of ways, we shall need a terminology to specify precisely what kind of an average is used. The discharge itself is normally measured once a day; in the course of a month it may vary quite appreciably. Let us specify by ‘instantaneous’ discharge such a daily measurement. The *mean* discharge will always refer to a mean of instantaneous discharges over a specified number of consecutive days; i.e. mean monthly, mean annual, or between two specified dates. The mean discharge averaged for the same calendar interval for a number of years will be referred to as the average mean discharge. Or we may take the median mean discharge, over a number of years, as a measure of central tendency. One can also define the average instantaneous, or median instantaneous, as the average, or median of discharge on the same day of the year for a number of years.

To obtain velocities at points on the river, other than metering stations, we must determine: (1) how to estimate the discharge for points on the river other than metering stations; (2) how to determine an area A appropriate to the particular discharge at any given point. Given values of D and A appropriate to each other, we then have a velocity measurement by equation (6).

In order to show how the first question can be answered, we have plotted in Fig. 4, for several metering stations, the average mean monthly discharges divided by the average mean annual discharge at the station. It will be seen that the entire river heaves up and down *en masse* in the course of a normal flood year. The average flood comes about one month later, and more abruptly in the Canadian headwaters than on the lower 700 miles from the mouth, in the United States. It can also be shown by plotting the average mean for a particular month against river mile, that the discharge

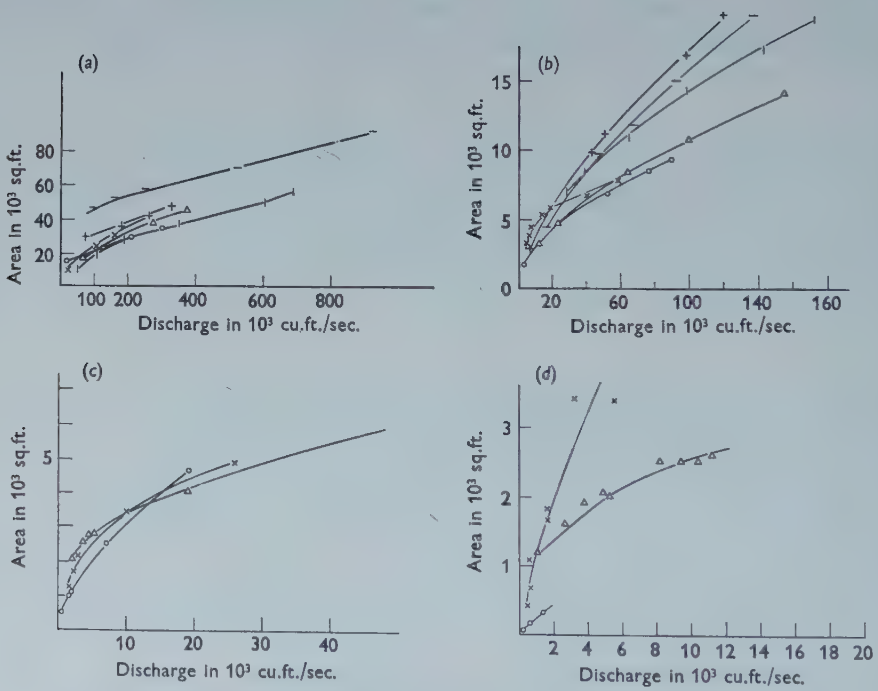

Fig. 3. Cross-sectional area *vs.* discharge in cu.ft./sec. for the Columbia River. Various dates of observation from 1910 to 1955.

(a)

	River miles
– Dalles	200
+ Hanford	364
△ Vernita	387
| Trinidad	440
○ G. Coulee	602
× K. Falls	705

(b)

	River miles
| Int. boundary	740
– Birchbank	765
△ Steamboat rap.	930
+ 12-mile ferry	940
× Downie Creek	960
○ Potlatch Creek	1000

(c)

△ Surprise rap.	1058
× Donald	1100
○ Nicholson	1120

(d)

△ Golden	1115
× Edgewater	1170
○ F.M. hot springs	1217

is a smooth and slowly varying function of distance from the mouth, except for discontinuities at substantial tributaries (see Fig. 5). Instantaneous and mean monthly discharges thus plotted are also very strongly correlated and in the same way, though one can detect the passage downstream of a specific flood crest by careful analysis of measurements very closely spaced in time.

The net consequence is that, given the discharge at a given time at one metering station, the simultaneous discharge for points as much as 400 miles away can be determined with a fair degree of accuracy. In practice we have used on our subsequent figures the discharge at Trinidad (R.M. 455) as our 'control' discharge, since this metering station is fairly in the middle of the course traversed by the salmon migration on the Columbia.

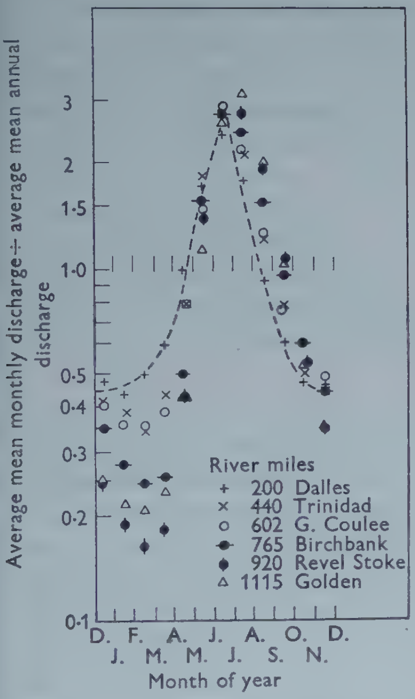

Fig. 4

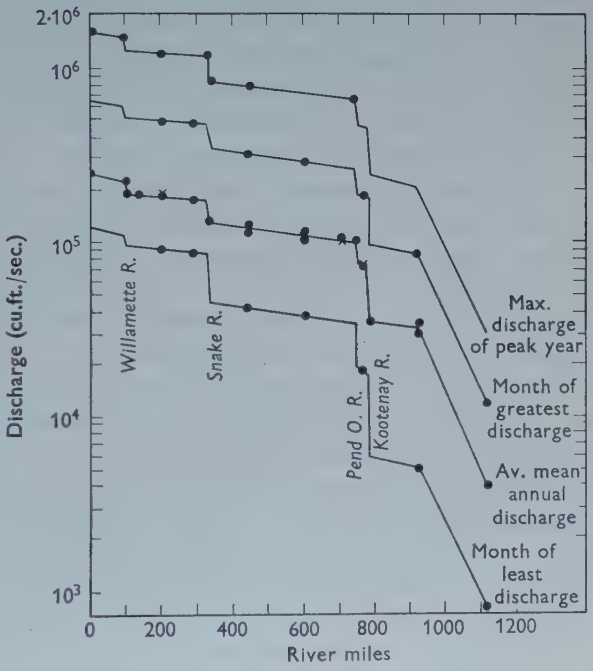

Fig. 5

Fig. 4. The ratio of average mean monthly discharge to average mean annual discharge for various metering stations on the Columbia River. (Line fitted to The Dalles data.)

Fig. 5. Average mean discharge in cu.ft./sec. *vs.* river mile from mouth of the Columbia river. The data correspond to the least and largest average mean monthly discharges of Fig. 4, the average mean annual discharge and the peak instantaneous discharge of 1894 (extrapolated by parallelism in Canada (R.M. > 800). Filled circles, U.S. Army Engineers (1948, 1933); crosses, USGS Water Supply papers.

Let us now answer the second question: how to determine the cross-sectional area appropriate to a given discharge. We first point out that this area is, at a given point on the river, determined by underwater and above-water ground contours of elevation above sea level, plus the elevation of the water surface above sea level. This latter elevation naturally varies, as given by the rating curve, with the discharge. Now there are two extreme conditions for which the water-surface elevation is well documented: (*a*) for flood conditions of large discharge, since this condition determines what places are likely to be safe; (*b*) low-water conditions of small discharge, which are also fairly well documented, since this condition determines the navigability of the river. Hence, if we can find a reasonable method of interpolation between these two extremes (using the discharge itself as independent or interpolation variable, as obtained under question 1) we can determine the area at any point where the contour maps are available.

Such an interpolation method is suggested by Fig. 3, which gives the discharge versus area for a number of metering stations. It will be seen that, at least in the first 700 miles from the mouth, the assumption that area is a linear function of discharge is a fair approximation (upper left plot of Fig. 3). It is also at sites in this 700-mile stretch that we had contour maps and water-surface elevations requiring this interpolation. Such sites were obtained from the U.S. Coast and Geodetic survey maps (1954, river mile 0 to 140) plus maps of dam sites, bridges and navigation projects in the Army Engineers 1948 report. The net result of this discussion is that we can obtain velocity measurements at quite a number of points, in addition to the velocities obtainable directly from the metering stations.

Additional, independent velocity measurements can be inferred from the times and places passed by diarists (Symons, 1882; Freeman, 1921) who have descended the Columbia in small boats. These have been corrected for the labours of the oarsmen; a correction from surface to average velocity (Marks, 1941) has been made, and, when plotted in Fig. 6, an estimated correction from the discharge at the date of observation to the discharge appropriate to Fig. 6.

We thus have velocity measurements from three sources of data: (1) directly from the metering stations; (2) indirectly from contour-depth maps, and interpolated discharge and water elevation data; (3) the corrected surface velocity measurements from the diaries. All of these velocities are plotted in Fig. 6 for discharges corresponding to the months of least (January, February) and largest (June, July) average mean monthly discharge indicated in Figs. 4 and 5, and the average mean annual discharge (approximately April and August). The velocity corresponding to the instantaneous peak discharge of 1894 is also given, since this flood has given a well-documented high water-surface elevation, and also represents an upper limit for velocity in most cases.

All of these measurements seem to be fairly consistent. In Fig. 6 arithmetic averages for 100-mile reaches are shown dotted, and these numerical values are used in computing the fuel requirement from equation (5).

There is, of course, considerable fluctuation in the individual velocities in Fig. 6. This fluctuation is taken into account in computing equation (5) by using the appropriate relations, computed from the data,

$$(V^2)_{\text{av.}} = 1{\cdot}12(V_{\text{av.}})^2,$$
$$(V^3)_{\text{av.}} = 1{\cdot}32(V_{\text{av.}})^3.$$

These fluctuations are for the most part real, since the cross-sectional area of the river does vary considerably in a distance of 100 miles whereas the discharge does not, except where the 100 miles contains a large tributary.

It will be noted that there seems to be a systematic discrepancy between the velocities inferred from Freeman's diary and the velocities from the Canadian water metering stations (R.M. > 800). This, we suspect, is due to selection of low-velocity sites for metering. Water-metering points are preferably chosen at a point where the water surface is reasonably calm and level, i.e. above a 'control' such as rapids. The

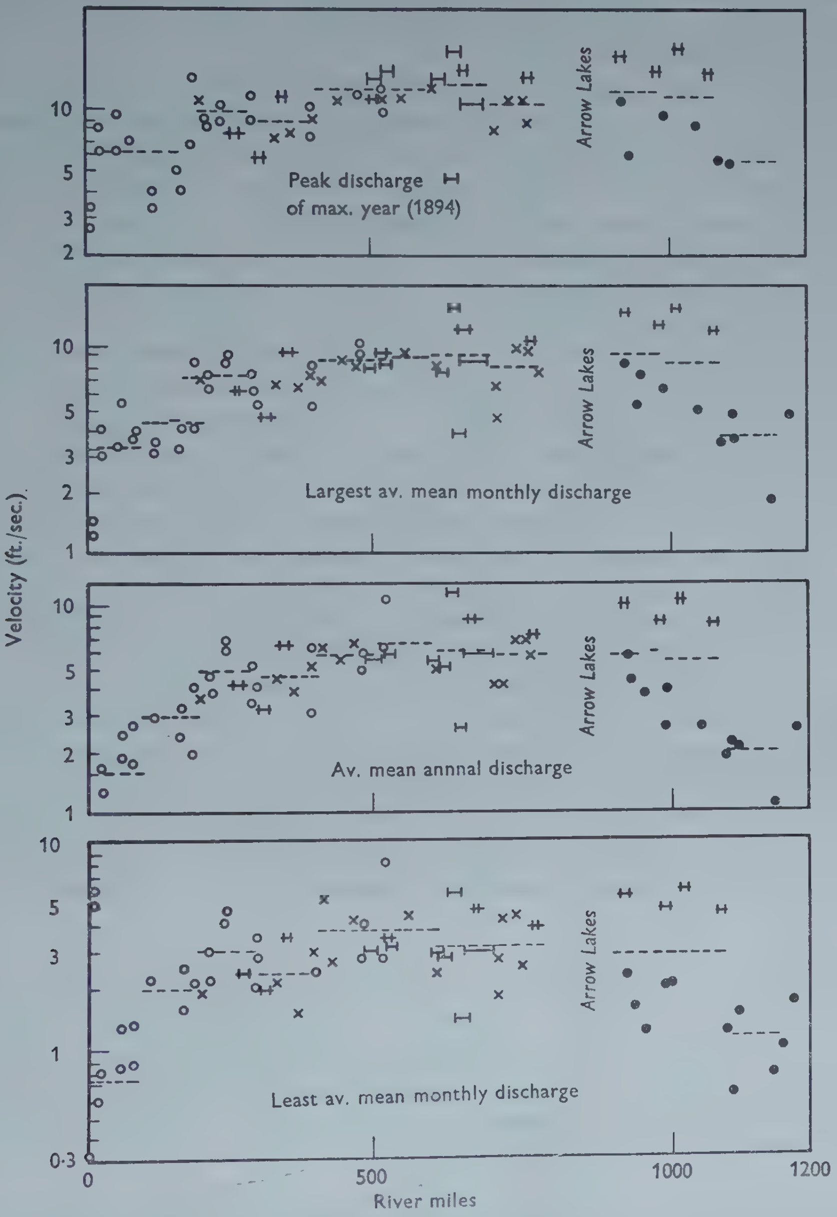

Fig. 6. Average velocity (= discharge/area) for the Columbia River for the discharges of Fig. 5. Pairs of points at the same river mile in open circle data correspond to cross-sections taken 1–2 miles apart, this being frequently the extent of the dam-site maps. The velocities refer to the river without dams. ○, USCGS and Army Engineers dam-site data; ×, U.S. Geological Survey Water metering points; ●, Research Board of Canada meter points; ⊢⊣, Symons; ++, Freeman; – – –, 100 mile average.

Canadian reaches are commonly described as the more swift and turbulent portion of the river; actually its swiftest portions were not traversed by Freeman, who had to portage or come down on lines.

The remaining reaches of Fig. 6 seem not to show any systematic discrepancies between the different types of data. We feel that the combined data of Fig. 6 gives a fair picture of the average velocities and fluctuations in velocity encountered by our vehicle, and the fish, in their ascent.

Fig. 6 can be used to check the order of magnitude of fuel requirement quoted in this paper by using a single velocity averaged for the entire length of a salmon run. Fig. 6 can also be used for a rough examination of cases other than those considered in this paper. It should be noted, however, that fuel consumption of our vehicles varies (in the neighbourhood of the optimum $V_F = \frac{1}{2}V_W$) as the square of the water velocity, so that such an averaging procedure as described above will slightly underestimate the fuel requirement as given by a more exact calculation.

For completeness one should describe how the velocity varies in a given cross-section (Corbett and others, 1943). The velocity at a metering site is determined by means of a small propeller-type meter, hung at various points in the cross-section; summation of velocities thus measured over the area gives the discharge. Considering velocity measurements on a typical vertical line, velocities 'on the bottom' (actually about 6 in. off the bottom) are about one-half the average velocity on the vertical. At a distance equal to 20% of the depth from the bottom, the velocity has arisen to the average value, and increases only slightly in the remaining 80% of the depth to the surface. This velocity profile is quite characteristic of flow in a broad and shallow channel with turbulent momentum transfer (Prandtl-Tietjens, 1934), which would appear to be a reasonable characterization of a river. In summary, except close to the bottom or shoreline, the actual velocity at any point in the river is of the order of the average over the entire section. Exceptions can occur where the channel is markedly curved or divergent (Goldstein, 1938), giving regions of back flow, but even in such cases the average velocity is still given by the discharge over the cross-sectional area. In our calculations we shall assume that, when in progress, our vehicles encounter the average flow, without seeking systematically either low- or high-velocity water. Whether real fish do likewise remains to be discussed.

THE SCHEDULE OF ASCENT FOR THE FISH

April to October is the season over which the spawning migrations occur. Over this interval the discharge varies by a factor of about 10, and water velocities by a factor of 2 or 3 (Figs. 4, 6). Hence, the fuel consumption of our hypothetical vehicles will be markedly affected by the season of their ascent, or schedule for arrival at the successive reaches of the river. We should pick these schedules to correspond with the schedules for real fish.

Such schedules can be read off from Fig. 7. In this figure the data points are as follows. At the mouth of the river, Bonneville and Rock Island, the horizontal lines with vertical bar represent major peaks in the weekly catch or count data on Columbia River salmon of Rich's analysis (Rich, 1942). In these data there are three principal peaks corresponding to the spring, summer and fall runs of chinook salmon.

The vertical bar represents the maxima of these peaks, the end-points the interval over which the count or catch fell to one-half its maximum value. The bar end horizontal lines in the neighbourhood of Kettle Falls correspond to reports as to when 'large numbers' of salmon appeared in the past (Bryant & Parkhurst, 1950). There is also a report at the headwaters (Golden, R.M. 1100) on the regular annual appearance of large, 40-lb. chinooks.

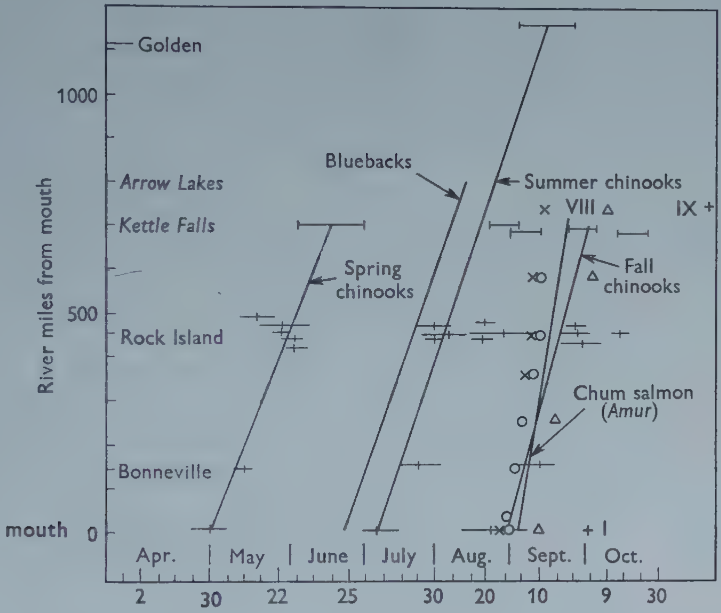

Fig. 7. The schedule of ascent for salmon on the Columbia and Amur rivers, river miles from the mouth *vs.* date. The numbers on the abscissa scale refer to week-end dates of Rich's original analysis (1942), since subsequent weekly fish counts have been published on this basis. The horizontal lines are data points for Columbia River salmon, as described in the text. The Roman numerals refer to Pentegoff's stations. I was at the mouth of the Amur, VIII was prior to spawning. At station IX, a short distance away (but 2 months later), spawning had begun. The data points for the Amur River are: (×) appearance of chum salmon, (○) beginning of commercial fishing, (△) end of commercial fishing, (+) end of run.

For determining a schedule of ascent, we have drawn straight lines by eye for the three principal salmon runs, and from these lines the date for the mid-point of each 100-mile reach was used to determine V_W for that reach, for the purpose of computing fuel requirements according to equation (5). The spring run of salmon is quite clear cut in this procedure. The distinction between summer and fall runs is not always as sharp as one might wish, but the straight lines certainly represent reasonable approximations to the schedule of actual ascent of fish. We have also drawn a line (data not given) for bluebacks ascending to Arrow Lakes (R.M. 800).

There is also, on Fig. 7, data points as given by Pentegoff, Mentoff & Kurnaeff (1928) for the various stages of the run of chum salmon up the Amur River, the schedule for this migration is also approximated by a straight line.

The following points concerning Fig. 7, in relation to the water velocities of Fig. 6, should be noted. Suppose we computed fish velocities from these data (the slope of an ascent line). This turns out to be about 1 ft./sec. for the Columbia River salmon, independent of the season of the year. This, of course, assumes a uniform rate of progress night and day—from biological observations, a most unfish-like behaviour. If we compare this velocity with the water velocities of Fig. 6, this fish velocity for Columbia River salmon is seen to be much less than the hypothetical optimum $V_F = \frac{1}{2}V_W$. Now the water velocity varies by a factor of 2 to 3 over the migration season (April to October), without appearing, from this very approximate data, to have any appreciable effect on the average velocity of the fish. This seems quite surprising, and we shall examine in a subsequent section with more accurate data the relationship between the observed velocity of water (as measured by discharge) and the rate of progress of fish upstream (see, for example, Fig. 9).

The data on chum salmon migrating up the Amur indicate a somewhat different relation between water velocity and implied fish velocity than that for the Columbia. Pentegoff's data (in which we have found some small errors) gives 69 km./day (= 2·6 ft./sec.) for the average water velocity over the entire distance of salmon run, and 50 km./day (= 1·9 ft./sec.) for the rate of progress of the fish. For these fish, in contrast to the Columbia River salmon, the average rate of progress is somewhat greater, instead of less, than the optimum $V_F = \frac{1}{2}V_W$.

The water velocity on the Amur, it will be noted, is considerably less than for the corresponding length of the Columbia River. Pentegoff's water velocities were apparently taken from the diary of a boat trip down the Amur in 1897. What correction was applied for the year (1921) of Pentegoff's observation is not known.

Fig. 7 is primarily intended to give a schedule for ascent of our hypothetical vehicle, rather than allow any firm conclusion about fish velocities, though it does, of course, give lower limits on their average values. In computing fuel requirements with equation (5), for vehicles corresponding to Columbia River salmon, we shall suppose they progress at the optimum $V_F = \frac{1}{2}V_W$ with intervals of rest (actually about two-thirds the transit time) sufficient to stay on schedule. For vehicles corresponding to chum salmon on the Amur we shall use just the numerical values quoted above for V_F and V_W. This is the simplest assumption for least energy consumption we can make concerning them.

CALCULATION OF REQUIRED *VS.* AVAILABLE FUEL

The dimensions and other specifications of our vehicles are given in Table 1, labelled by the name of the equivalent fish which they are intended to approximate. The fuel requirements computed from these specifications by equation (5) are listed in table 3. However, two additional fuel increments have been included in Table 3. The computations were carried out for an average flood year (as in Fig. 4), but in some years the flood is much greater than the average. We have estimated, from the measured discharges and velocities associated with a large flood, that an increment of 25 % in the computed fuel for an average year would be sufficient to cover five-sixths of all floods. Hence, a 25 % flood reserve has been added to the fuel requirement computed from equation (5) for an average year.

Secondly, it was pointed out that, at least for the case of the Columbia River, about two-thirds of our vehicle's transit time would be spent resting, presumably in still water. Hence it seemed reasonable to make an estimate of the fuel consumed during this resting. This fuel consumption we have estimated from the CO_2 generation and O_2 consumption of known weight of (quiet) salmon, given in the report of Fish & Hannevan (1948). It amounts to 0·7 g. of fat per 100 g. of fish, for the 700-mile migration from the mouth of the river to Kettle Falls, and corresponding amounts for other distances. This small increment is included in the total fuel requirement given in Table 3.

Calculation of the fuel actually consumed by real fish requires a knowledge of the weight and chemical composition (fat, protein, water) of the fish on entering the mouth of the river from the sea, and on arrival at the spawning grounds. These latter data are given prior to spawning, since a good deal of time and energy is expended in this activity. A summary of these data is given in Table 2; concerning it we make the following comments.

Data for chum salmon in the Amur are taken directly from Pentegoff's paper, who gives detailed analyses and weights for the various stages of migration. For the Columbia River salmon we have taken average figures from a variety of sources (Shostrom, Clough & Clark 1924; Greene, 1919, 1921). These average figures indicate that the majority of the fuel consumed is obtained from fat in the muscle tissue, which muscle tissue we have taken as two-thirds the body weight at the mouth of the river. Since the percentage of fat in this tissue fluctuates so widely, according to Shostrom's data, we have taken the fat portion of muscle tissue as 18 ± 10 % and carried the effect of the ± 10 % fluctuation throughout the calculation. We have assumed the entire body weight shrinks during the migration to 80 % of its value at the mouth, the body tissue being partly consumed and partly going into the reproductive products. The net protein which disappears is rated at 0·44 the fuel value of fat. The residual part of the body weight which is neither muscle tissue nor reproductive products, we have assigned a fuel value two-thirds the value for muscle, at the river mouth, and one-third at the spawning grounds. These figures are estimates based on chemical analyses of body components given by Pentegoff and Greene. We feel these figures do not underestimate the net fuel which might be contributed by this residual.

On comparing the fuel required by our vehicle with the fuel available to real fish, in Table 3, we can make the following observations. The first three entries in column 3 of Table 3 show a decreasing fuel requirement with later season of ascent, a simple consequence of the successively lower water velocities encountered.

Other factors being equal, smaller vehicles require more fuel per 100 g. of body weight than large ones. Both seasonal and size factors conspire to make the vehicle similar to bluebacks require relatively the most fuel, since these fish both are small and ascend (see Fig. 7) during the peak of the flood. The combined advantage of large size and avoiding flood water is shown by comparing big chinooks to the headwaters of the Columbia (1200 R.M.) with spring chinooks to Kettle Falls (700 R.M.).

Except for chum salmon ascending the Amur, and fall chinook ascending the Columbia, the fish have out-performed (used less fuel than) the best designed vehicle we could reasonably imagine. However, the discrepancy between the performance of our vehicles and real fish is not large except in the case of blueback salmon. These, we note, are the smallest fish considered.

Table 1. *Specifications of rigid vehicles equivalent to fish*

Species	Mass (g.)	Total surface area (cm²)	'Length', snout tip to narrowest point of tail (cm.)
Blueback (*Oncorhynchus nerka*)	1,350	1,060	41·8
Chinook (*O. tshawytscha*)	10,000	3,340	66·2
Large chinook	20,000	5,300	83·5
Chum (*O. keta*)	4,000	1,830	56·0

Table 2. *Determination of fuel available to real fish*

Columbia River chinooks and bluebacks

River mouth		Arrival at spawning grounds (prior to spawning)	
Mass	100 g.	Mass	80 g.
Muscle	68 g. 18 ± 10 % fat 18 % protein 74 % water	Muscle	80 × 0·50 g. 3 % fat 16 % protein 81 % water
		Reproductive parts	80 × 0·15 g. 14 % fat 25 % protein 61 % water
Residue	32 g. ⅔ fuel value of muscle	Residue	80 × 0·35 g. ⅓ fuel value of muscle
Fuel available, equivalent g. of fat	23·1 ± 8·9		7·9

Difference = net fuel available for propulsion = 15·2 ± 8·9 equivalent g. fat per 100 g. mass.

Amur River chum salmon

River mouth		Arrival at spawning ground	
Mass	100 g.	Mass	92·5 g.
Muscle	68 g. 12·2 % fat 20·5 % protein 67·3 % water	Muscle	92·5 × 0·54 g. 3 % fat 18·9 % protein 81 % water
		Reproductive parts	92·5 × 0·17 g. 14 % fat 25 % protein 61 % water
Residue	32 g. ⅔ fuel value of muscle	Residue	92·5 × 0·29 g. ⅓ fuel value of muscle
Fuel available, equivalent g. fat	19·1		10·5

Difference = net fuel available for propulsion = 8·6 equivalent g. fat per 100 g. mass.

PERFORMANCE DIFFERENCES OF VEHICLES *VS.* FISH

We shall now examine more closely the assumptions and approximations we have made concerning our vehicle, relative to the behaviour and properties of real fish. We shall divide the following discussion into two parts: (1) Evidence on the behaviour of fish permitting the computed fuel consumption of our vehicle to be decreased—

Table 3. *Comparison of fuel required* vs. *available*

	Distance (R.M.)	Initial mass (kg.)	Vehicle fuel requirement (g. fat per 100 g. of initial mass)	Fuel available to fish (g. fat per 100 g. of initial mass)
Spring chinook	700	10	33·5	15·2±8·9
Summer chinook	700	10	24·2	15·2±8·9
Fall chinook	700	10	14·3	15·2±8·9
Large (summer) chinook	1200	20	28·2	15·2±8·9
Bluebacks	800	1·35	75·2	15·2±8·9
Chum salmon (Amur)	750	4·0	8·0	8·6

i.e. making the performance of our vehicle more nearly that of real fish. (2) Evidence which leads the other way, giving an even better performance by fish over vehicle than the above comparison already indicates.

The first point concerns the average velocity of the water in which fish swim. Do they, as we have supposed for our vehicles, oppose the average flow, or do they systematically seek out low-velocity water? Correspondence and conversation with biologists familiar with these fish give the impression that the fish (and especially bluebacks) do seek out low-velocity water near the bottom and edges of the river. As mentioned in our discussion of the distribution of the velocities in a cross-sectional area, within 6 in. of the bottom the velocity is one-half the average. Moving so close to the bottom would cut fuel consumption to roughly $(\frac{1}{2})^2 = \frac{1}{4}$ that computed for no-velocity selection. Except for the 'blueback vehicle', this would reduce the fuel consumption of our vehicles to well below the fuel available to real fish.

An exception to this preference for low-velocity water should be made for the neighbourhood of dams and ladders. Here, judging by the description of fish behaviour as given in the Bonneville Reports (U.S. Army Engineers—annual publication) the fish seem to be attracted by high-velocity water. Presumably this is a special situation to which the fish react in a special way, since only by seeking out moving water can a passage over the dam be found.

The second point concerns the use of the turbulent, as opposed to a laminar boundary-layer friction coefficient. Is it possible, despite the observations on dead fish, for a live and actively swimming fish to achieve a laminar flow, with no separation, over its entire body surface? The observations of Gray (1933, 1936) together with the theory of how drag arises, suggest that it might indeed be possible. Gray clearly showed that a swimming fish sends a travelling wave down its body, and that the propulsive force is applied over the entire body surface, not only with the tail. Now this means that the fish is putting momentum into the fluid over his entire surface, which surface is precisely the site where the loss of momentum by the fluid occurs, which loss is responsible for the drag. Hence, this swimming mechanism (travelling waves) returns to the boundary layer just the momentum which the fluid is losing, which loss would (for a rigid vehicle) ultimately result in a laminar to turbulent transition or separation, or both. It is well known that either replacing the momentum loss of the boundary layer by blowing, mixing, boundary motion parallel to the flow, or by removing the low momentum fluid (suction) will prevent both separation and (in some cases) a laminar-turbulent transition (Goldstein, 1938). This may be precisely what the travelling waves down the fish's body (boundary motion at right angles to the flow) accomplishes. The gills may also play a role in this process, since they also add momentum to the boundary layer (Breder, 1926).

The third point concerns the intake of energy, by the vehicle (or fish) from the river current. We specified that our vehicle, like the fish, should take in no fuel en route. But it does not necessarily follow that the energy intake (from the river flow) must also be zero. There is, of course, considerable energy in the turbulent velocity fluctuations in the river current, and if the fish could take momentary advantage of these, they would provide a source of energy for propulsion. A precedent for such utilization of turbulent energy for propulsion is given by the Betz-Knoller, or Katzmayr effect (Kuchemann & Weber, 1953). This effect, in the simplest terms, is similar to 'tacking' by a sail-boat, but the direction of the current changes (the turbulent fluctuation) rather than the direction of the vehicle, as in the case of a sail-boat. Whether fish can, in fact, utilize this effect is not known.

We should also like to point out that from a strictly engineering standpoint, there is no objection to utilizing the energy of the river current, whether steady or turbulent, to reverse the direction of the chemical reaction which normally provides propulsion. For man-operated vehicles, anchored to the bottom, recharging a storage battery from the propeller driven as a turbine would be the simplest example.

The biological equivalent of 'recharging the battery' would be the reversal of the chemical reaction associated with muscle contraction, with work done on the muscle. It is known that such work can store up appreciable chemical energy for a *single* muscle contraction, as experiments on frog and human muscle have amply demonstrated (Hill, 1960). The extent to which fish can utilize such a process to extend their effective fuel supply is, of course, quite conjectural. Such a process could also be interpreted as dynamical 'damping' of boundary-layer fluctuations, which has been demonstrated as a means of boundary-layer control (Kramer, 1960).

The above discussion summarizes the arguments which might tend to make our vehicles more fish-like and at the same time consume less fuel. There are, however, cases where we may be compelled to conclude the opposite—more fuel consumption. As a possible example, Fig. 1 shows that salmon, and notably bluebacks, ascend 800 miles to Red Fish Lake against currents which in flood times are probably swifter than those of the Columbia. A second example is given by Greene (1919), who found salmon with 10% muscle fat on the Snake River, 700 miles from the sea. Presumably these fish have out-performed those on the Columbia by a considerable margin, since they had covered 700 miles and had (presumably) over one-half their fuel still remaining. Still another example would be (for our vehicles) ascent in an exceptionally large flood. However, this example illustrates a significant difference between our vehicles and real fish. Large floods are commonly believed, with good reason, to render passage easier for the fish, but simply add to the fuel requirement of our vehicles (see discussion of Figs. 12 and 13).

From an engineering viewpoint, the ability of the salmon to ascend such great distances with so little fuel consumption presents a near paradox. If we accept our basic assumptions as generally typical and, further, for the bluebacks, allow that they reach the spawning grounds by selecting out the lowest velocity waters and are characterized by a laminar-flow drag coefficient, the performance specifications of the fish (as represented by our vehicles) are still impressive. The tail of the fish is the best propeller, the body chemistry of the fish is the best engine, and the flow characteristics of his body are better than any observed from dead fish. Moreover, the fish shows great competence, both in time and place, in his selection of optimum water velocity under constantly changing environmental conditions from ocean to spawning ground. The maintenance of such top performance specifications under such varied conditions is an astounding engineering feat.

THE RELATION OF DISCHARGE TO TRANSIT TIME

In this section we wish to examine more closely a question briefly referred to earlier. Is there any observed relation between the time it takes fish to traverse a given reach of river and the velocity of the water in that reach? It should be noted that in the present discussion the words 'fish velocity' mean the distance between dams divided by the time interval of transit between them. This velocity is not the 'fish velocity' used in computing the fuel requirement according to equation (5), for the Columbia River.

Let us imagine that the Columbia River actually consists of two rivers, a river of water flowing down, with discharge measured in cu.ft./sec., and a river of fish flowing up, whose 'discharge' is measured in numbers of fish per day.

Fig. 8 gives a typical plot of 'fish discharge' at the Bonneville and Rock Island dams (or metering stations) taken from the U.S. Army Engineers Bonneville Report for 1951. It will be seen that, generally speaking, a peak at the lower dam is followed by a similar peak at the upper dam a few weeks later. If we identify these peaks with the same fish, we then have a measure of the average fish velocity defined as (distance BV to RI)/$(T_{RI}-T_B)$ when T_{RI} and T_B are the dates of the peaks at Rock Island and Bonneville, and hence $T_{RI}-T_B$ the transit time between them. This property of the fish river should be contrasted with that of the water river, Fig. 4, for which the discharge increases and decreases almost simultaneously at the various metering stations.

Such an identification of fish is clearest and simplest for the case of the bluebacks, which pass up the river each year in a single, well-defined 'shock wave'. We have chosen to use the median of the blueback count as a measure of the date of passage of fish over the dam, and have plotted in Fig. 9(*a*), (*b*) these median dates, T_B, T_{RI}. The difference $T_{RI}-T_B$, or interval of transit between dams, is given in Fig. 9(*c*). The abscissa is the mean discharge at Trinidad for the month of July. The data at Bonneville cover the years 1938–54; at Rock Island 1933–54 (Fish & Hannevan, 1948; U.S. Army Engineers Bonneville Reports).

It should be especially noted that the mean discharge has been taken in all these plots for the month of July for each year; actually also the month of transit in most cases, and has *not* been taken on a date given by T_B, T_{RI} or the 'mean median' date of transit $\frac{1}{2}(T_B+T_{RI})$ for each year. The reason we have not made any of the above 'obvious' choices is that we are interested in the relation of discharge (of river) to transit time and arrival date of fish. Were we to use instantaneous discharges on dates different in each year, we would find our conclusions distorted by the fact that the river discharge itself is systematically declining in July; i.e. random dates in July would have a strong *negative regression* of date on discharge *at the same date*.

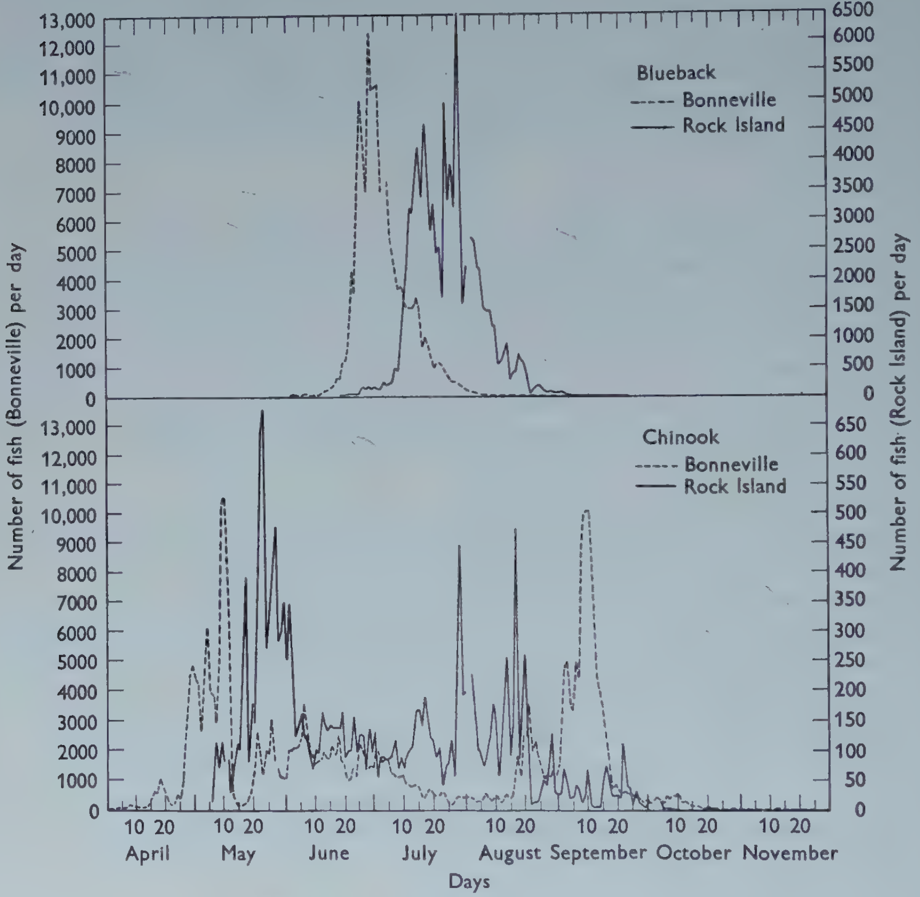

Fig. 8. The number of fish per day counted over the Bonneville Dam (R.M. 140) and the Rock Island Dam (R.M. 440) for the year 1951. Variations in the attrition between Bonneville and Rock Island can be noted by comparing data with the right- and left-hand scales.

Examination of Fig. 9 will show that, in fact, the arrival date at Bonneville is uncorrelated with discharge (Fig. 9(*a*)), while at Rock Island the larger the discharge for July the later the arrival date (Fig. 9(*b*)). Note that this latter regression is precisely the opposite effect to be expected from the behaviour of the river alone, for which the earlier the date in July the larger the discharge. The transit time, or the difference between Rock Island and Bonneville arrival dates, also increases with increasing July discharge (Fig. 9(*c*)).

Hence, we can state, unambiguously, that for bluebacks a large discharge does affect both arrival date at Rock Island, and the transit time between dams, making them respectively later and larger. All this seems quite reasonable—a large discharge implies a large water velocity, which slows down the fish between Bonneville and Rock Island but not appreciably below Bonneville. The arrival date at Bonneville seems unaffected by discharge. Below Bonneville the water velocities are in general smaller than above —there is, in fact, a small tide in the river below Bonneville.

In the case of the chinook salmon, examination of the data of Fig. 8 shows a rough correspondence between the numbers of fish per day at Bonneville and at Rock Island a few weeks later, though the correspondence is by no means so clear-cut as in the case of bluebacks.

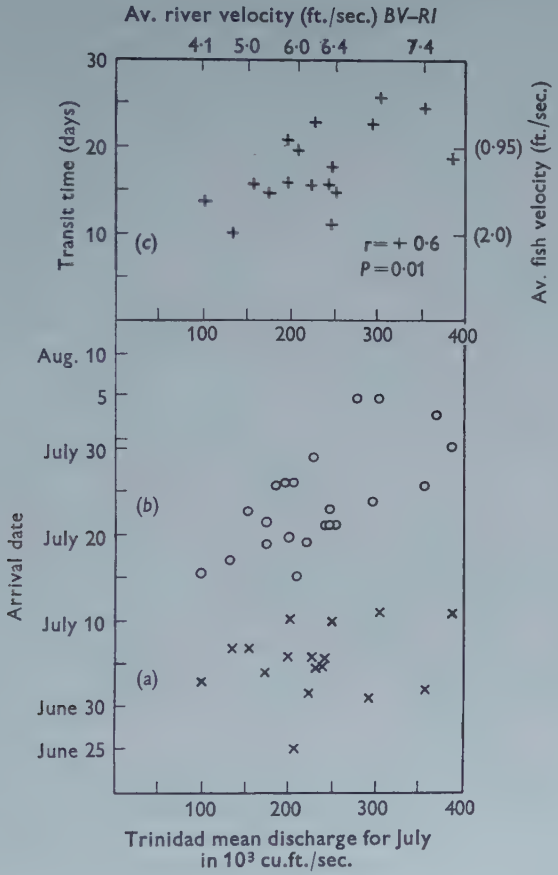

Fig. 9. Median arrival dates for bluebacks at Bonneville (*a*) and Rock Island (*b*), and the difference, or transit time (*c*) plotted against Trinidad mean discharge for July. The upper abscissa scale gives the average water velocity between Bonneville and Rock Island (from Fig. 6); the ordinate scale on the right gives the average fish velocity as determined from distance between dams divided by transit time. +, Transit time, $T_{RI} - T_B$; ○, *RI* arrival date, T_{RI}; ×, *BV* arrival date T_B.

We have endeavoured to analyse the chinook counts in order to obtain their transit time between the dams as follows. A tracing was made of the count at one dam and superimposed on the other. Attention was focused on, say, a 30-day interval at one dam, and the tracing was slid till a sufficiently significant match was found with an equal interval on the other plot. The median dates, T_B and T_{RI}, for the fluctuations on the two plots were then recorded and plotted against each other (Fig. 10). This process was carried out for the years 1938–54 for which counts exist for both dams, and amounts to graphical computation of the interval of maximum lag correlation. This process is admittedly somewhat subjective, but it does provide a measure of the transit time between dams. As can be seen from Fig. 8, the correlation between fish counts in the spring at the two dams is much clearer than in the fall. Also, the fraction of the fish over the lower dam (Bonneville) which also passes over the upper one (Rock Island) varies considerably throughout the year.

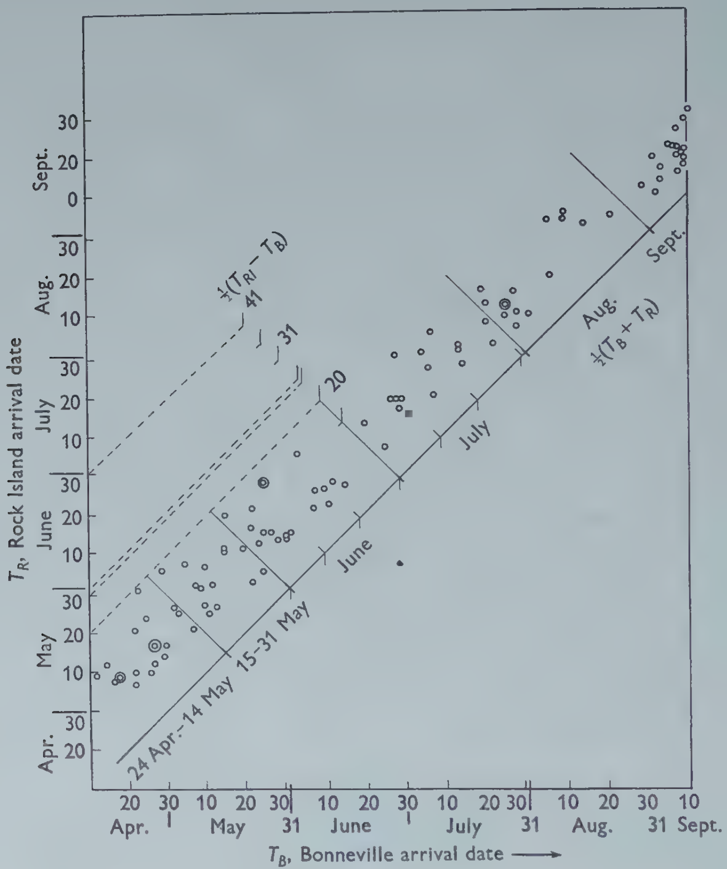

Fig. 10. Maximum correlation arrival dates for chinook salmon at Bonneville and Rock Island. The co-ordinates of the data points are the median dates of fluctuations in the Bonneville and Rock Island counts (as in Fig. 8) which could be matched to each other. The tilted co-ordinates give the mean of such Bonneville and Rock Island dates, $\frac{1}{2}(T_B+T_{RI})$, and one-half the transit time, or $\frac{1}{2}(T_{RI}-T_B)$, but units of one day measured parallel to these co-ordinates are not of equal length, as shown by the angled 10-day markers. The blocks of data marked off in $\frac{1}{2}(T_B+T_{RI})$ have their median transit times and dispersion of transit time plotted in Fig. 11.

The data points on Fig. 10 were then divided into class intervals of $\frac{1}{2}(T_B+T_{RI})$ (marked on the diagonal line), containing approximately equal numbers of data points. For each of these classes the transit time $T_{RI}-T_B$ was then plotted against the mean discharge at Trinidad for the time interval of the class for that year (same interval for each year). Two such plots of transit time against discharge are given in Figs. 12

and 13. From such plots, for each interval marked on Fig. 10, there can be obtained, with fair accuracy, the median mean discharge and the dispersion of mean discharge for the class interval in $\frac{1}{2}(T_B + T_{RI})$, fixed for all years covered by the data. One can also obtain the median transit time $T_{RI} - T_B$ and the dispersion of transit time for the particular (fixed) class interval of $\frac{1}{2}(T_B + T_{RI})$. These medians and dispersions of both mean discharge and transit time (marked by arrows on Figs. 12, 13) are plotted in superposition in Fig. 11. It is at once apparent that they all fluctuate together. The median mean discharge, a simple property of the river alone, rises and falls in the normal flood pattern throughout a year (as in Fig. 4); the dispersion of the mean discharge is largest when the median mean discharge is largest in June and July. Precisely the same relation obtains for the median of the transit time, and its dispersion, both of which increase and decrease together as the year progresses.

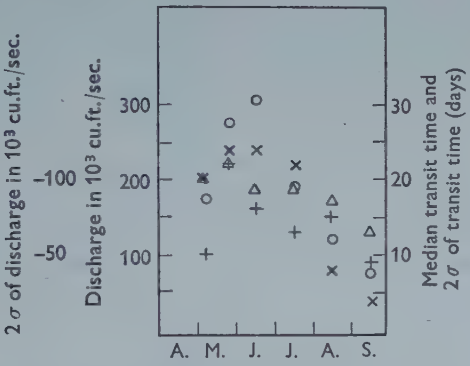

Fig. 11. Median of mean discharge, median of transit time and their (double) dispersions plotted against time of the year. The double dispersion 2σ is determined by taking the symmetric range around the median containing two-thirds of the data points (the double arrows of Figs. 12, 13). This choice provides a good measure of the root mean square deviation for a normal distribution, but does not require a normal distribution in order to have a simple statistical interpretation. ○, Discharge median; ×, discharge 2σ; △, transit time median; +, transit time 2σ.

Thus we can say that as a 'statistical' year progresses, for 'average instantaneous' discharge changing with time in this year, the general effect of a large (June) discharge (with large dispersion) on the fish is to slow them down (increased median transit time) and to increase the dispersion of the transit time.

The situation may be quite different when we examine the relation between mean discharge for a fixed class interval or season of the year, and the corresponding transit time, as in Figs. 9, 12 and 13. In Fig. 12 it will be seen that a mean discharge larger than the average mean discharge is associated with a decreased transit time (negative correlation), while in Fig. 13 precisely the opposite situation obtains. Here a larger mean discharge than 'average' gives a larger than average transit time (positive correlation). Fig. 13 gives a result similar to that for the bluebacks (Fig. 9).

Examination of the successive plots, from April to September (using the data of Fig. 10), gives the impression that the correlation ellipses tend to 'roll over' from before to after the flood, in the sense that the correlations are negative before the flood peak in June and positive thereafter, as Figs. 12 and 13 illustrate. Numerical values of the correlation coefficients are given in Table 4. While the significance levels are not high by most biological standards, we believe that the effect is genuine. Analysis of counts on silver salmon and steelhead trout gave similar results to those obtained for the bluebacks and chinooks (Osborne, 1959).

The negative correlation of transit time with mean discharge at a fixed season before the flood maximum, is perhaps one way of expressing a fact concerning salmon already well known; i.e. a tendency for fish to stop and wait for high water before continuing a migration ascent. In some cases the high water drowns out insurmountable falls and steep rapids. The negative correlation states that the larger the discharge the less the waiting, or smaller the transit time.

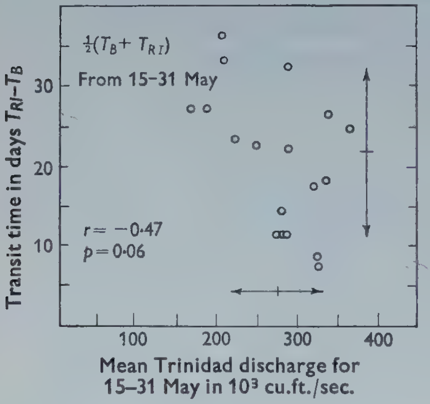

Fig. 12

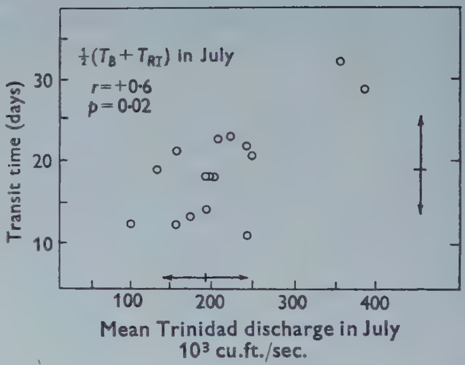

Fig. 13

Fig. 12. Transit time between dams of chinook salmon *vs.* mean Trinidad discharge for the interval 15–31 May.

Fig. 13. Transit time between dams of chinook salmon *vs.* mean Trinidad discharge for the month of July.

The positive (negative) correlations of transit time with mean discharge at a fixed season after (before) the flood peak, imply a small fuel saving which is deserving of mention. Thus, if a navigator is encountering a head wind which he knows will get stronger, then it saves fuel to speed up a little to reach his destination, whereas if he is encountering a head wind which it is known will get weaker, it saves fuel to slow down. Such behaviour is in agreement with the observed plus and minus correlations for fish in the Columbia. The actual fuel saved is relatively small. We have estimated

Table 4. *Correlation coefficients of transit time with mean discharge for a fixed interval*

Interval	*r*	*P*
24 April–14 May (chinooks)	−0·3	0·2
15–31 May (chinooks, Fig. 12)	−0·47	0·06
June (chinooks)	+0·25	0·28
July (chinooks, Fig. 13)	+0·6	0·02
July (bluebacks, Fig. 9)	+0·6	0·01
August (chinooks)	+0·35	0·30
September (chinooks)	0	1·0

The figure for *P* gives the probability of observing, with two uncorrelated normal variables, a correlation at least as large (of either sign) as that actually observed.

the effect of this correlation to reduce fuel requirement by about 10% below that for the constant velocity schedules (straight lines) of Fig. 7, for the case of the very largest floods, such as appeared in 1894 and 1948. Ascent of our vehicles in these floods would require about 50% more fuel than for an average year; i.e. about twice the allowed 'flood reserve'.

POSSIBLE FUTURE EXPERIMENTS

In the preceding discussion we have compared the fuel consumption of a hypothetical fish-like vehicle with the actual fuel available to real fish. The two figures agreed in order of magnitude, though the comparison indicated that the performance of the fish is in all probability superior to that of our best-designed vehicle.

Let us make the comparison in a slightly different way. Suppose one were to equate the actual fuel consumed by real fish with the theoretical fuel requirement of equation (5) and solve this equality for the undetermined coefficient C_f/N_1N_2. This would determine the effective C_f/N_1N_2 which the fish, considered as a rigid vehicle, must achieve in order to perform as well as he does.

The numerical values so obtained would be (except for bluebacks) of the order that we assumed initially; i.e. $C_f/N_1N_2 = \{0{\cdot}005/(0{\cdot}3)\,(0{\cdot}8)\} = 0{\cdot}02$.

There are, however, other ways of determining this coefficient. Let us imagine a fish swimming in a closed and recirculated water tunnel, with measured values of O_2 consumption and CO_2 generation. This would determine the energy consumption, and from the dimension and velocity of fish and the water, one could also determine, just as above, the value of the effective C_f/N_1N_2. Wohlschlage (1954) has performed experiments of just this type, and we have analysed his data, obtaining values of C_f/N_1N_2 approximately 100 times greater than those obtained as above for salmon. It should be noted, however, that Wohlschlage's fish were small ($\sim$ 200 g.), swimming slowly (1 m./min.) in water only a few degrees centigrade above freezing. Thus, the results of Wohlschlage are not strictly comparable with experiments on salmon; we mention them to show that the experiment is practicable, and give the order of magnitude of the conclusion.

We also believe that extended experiments of the above type would permit separate measurements of C_f and the product N_1N_2. Let us suppose, in the above experiment, the kinetic energy content of the momentum-free wake of the fish were also measured (Townsend, 1956). This would determine N_1N_2, or the fraction of total energy consumption (from O_2, CO_2 measurements) going into kinetic energy. Hence, C_f could also be determined. For a 20-lb. chinook swimming at 8 ft./sec. we estimate the velocity fluctuation in the wake immediately behind the fish at 5–10% the swimming speed. Thus, this experiment seems practicable, though perhaps not easy. One requirement is that the fish swim steadily under controlled conditions, a situation which can be realized experimentally using lights and electric potentials (Paulik & De Lacy, 1958).

SUMMARY

The annual spawning migration of Pacific Coast salmon up the Columbia and Amur rivers has been analysed as an experiment on the maximum range of an unrefuelled vehicle, since the fish do not eat during this migration. The fuel (primarily

fat) available to real fish is compared to that computed for an equivalent rigid vehicle with the following performance specifications which may be regarded as optimum ones for marine vehicles.

1. A choice for the speed of ascent against the average river current corresponding to the least fuel consumption, without seeking systematically either low- or high-velocity water.
2. A drag coefficient corresponding to a turbulent boundary layer with no separation.
3. An overall efficiency of 24 % for converting the heat of combustion of the fuel to directed kinetic energy in the wake. Under these assumptions, the vehicles usually require slightly more fuel than the corresponding fish which they are intended to approximate; in the case of blueback salmon, the vehicle requires about five times as much. One, therefore, concludes that the fish have a performance superior to that of the best engineered rigid vehicles, as defined and operated under the above three specifications. Suggested explanations for the apparently superior performance of the fish are:

(1) a deliberate seeking, by the fish, of low-velocity water close to the bottom or shoreline;
(2) the ability of the fish to maintain a laminar rather than turbulent boundary layer;
(3) the ability of the fish to extract energy from the turbulent velocity fluctuations of the river.

Analysis of salmon counts over the Bonneville and Rock Island dams on the Columbia River shows that the average velocity of the fish upstream is influenced by the velocity of the water downstream in the following peculiar fashion, depending upon whether one considers water-velocity variations through the course of a year, or variations at a fixed season of the year, from one year to the next. The general effect of the water velocity as it varies through a year, is to slow the fish down when the water velocity is large (at the June flood peak) and to permit faster ascent when the water velocity is less, in both preceding and following months. But at a *fixed* season of the year, prior to the flood peak in June, relatively high-velocity water speeds the fish up. At a fixed season after the flood peak, relatively high-velocity water slows them down. These relationships are in agreement with other biological observations and with an analysis of navigation for minimum fuel consumption.

Data and methods are given permitting a calculation of the average water velocity in the Columbia River, from the mouth to the headwaters, for any season of the year.

It is a pleasure to acknowledge the abundant advice and co-operation of the Staff of the Fish and Wildlife Service. Particular thanks are due to Mr Ralph P. Silliman, who provided the author with the biological background he so badly lacked on this problem, and Mr John Clark of the Bureau of Commercial Fisheries who subjected the manuscript to a most critical and comprehensive review. The author is also indebted to Wendell E. Smith of the State of Washington Fisheries Research Center for enlightening correspondence.

Unpublished data on river flow were provided by the Water Resources Board of the U.S. Geological Survey, Washington, DC. and by the Water Resources Division, Department of Northern Affairs and National Resources, British Columbia, Canada, to whom thanks are also extended.

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THE RESPONSES OF CERTAIN MYSIDS TO CHANGES IN HYDROSTATIC PRESSURE

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(*Received 7 December* 1960)

INTRODUCTION

Hardy & Paton (1947) obtained results from experiments in the sea on *Calanus finmarchicus* (Gunn.) which suggested that the vertical migration of this species might be affected by the change of pressure with depth. This prompted research on pressure effects and, since then, responses to changes of pressure by various animals have been observed in the laboratory by Hardy & Bainbridge (1951), Knight-Jones & Qasim (1955) and Baylor & Smith (1957). The present work is the first account of the pressure responses of mysids. It also appears to contain the first published demonstration that the behaviour of an animal may be affected by the pressure changes due to tidal rise and fall, although different responses recorded by Prof. E. W. Knight-Jones (personal communication) for the pycnogonid *Nymphon* may well have a tidal significance in nature.

MATERIAL AND METHODS

The mysids used were adult and immature specimens of *Schistomysis spiritus* (G. O. Sars) and *Siriella armata* (Milne Edwards) and adults of *Praunus flexuosus* (Müller) and *P. neglectus* (G. O. Sars), all collected by D-net in Port Erin bay. Recently hatched specimens of *Schistomysis spiritus*, released from the parents in the laboratory, were also used.

In the experiments a transparent Perspex observation tank 24 in. long and with a 2 in. square cross-section was used. An adjustable mercury column in transparent P.V.C. tubing communicated with the tank through an inlet in one side, and one of the end-plates could be removed to introduce sea water and animals. The tank could be held either vertically or horizontally and could be illuminated from above, below or from the side, the light always passing along the long axis of the tank. The light source used was either a projector lamp, giving a parallel beam slightly wider than the tank and an intensity of about 1000 lux, or a microscope lamp giving a narrower beam of about 850 lux. The same behaviour was exhibited whichever of these two light sources was used.

Usually between ten and thirty animals were used in each experiment and the numbers in each quarter of the tank were recorded at intervals; but occasionally the track of a single animal was observed.

Observations were made both on normal animals and on others without statocysts. The latter were obtained by first narcotizing with propylene phenoxetol and then removing the inner rami of the uropods, in which the statocysts are situated.

In the experiments conducted in darkness, observations were made by silhouetting the animals against a red light which was switched on only for the few seconds required to record the distribution in the tank.

In all the experiments the animals had been adapted to the lighting conditions of the experiment for at least 10 min. before recording was started.

Pressures referred to in the results are all in excess of atmospheric pressure and are recorded in millibars. One millibar equals the pressure produced by approximately 1 cm. of sea water.

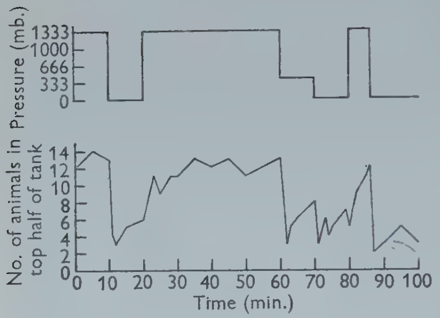

Fig. 1

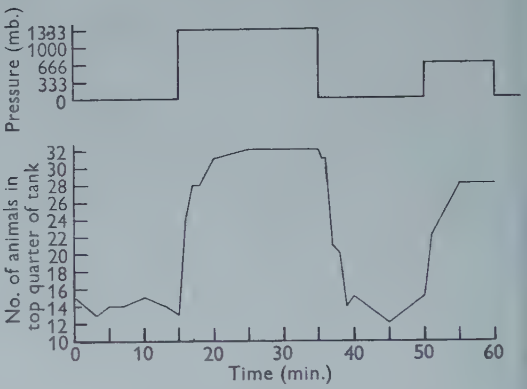

Fig. 2

Fig. 1. Distribution during pressure changes of fifteen specimens of *Schistomysis spiritus* in darkness.

Fig. 2. Distribution during pressure changes of thirty-two specimens of *Schistomysis spiritus* in overhead light.

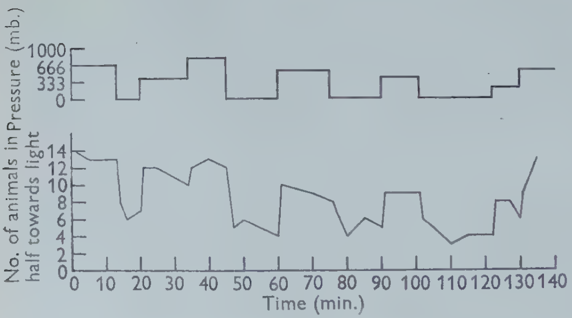

Fig. 3

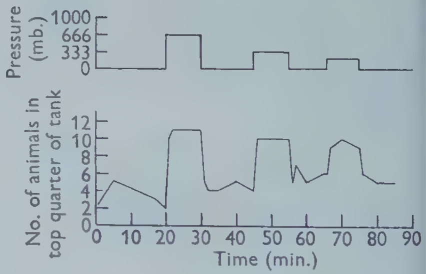

Fig. 4

Fig. 3. Distribution during pressure changes of fourteen specimens of *Schistomysis spiritus* in horizontal light.

Fig. 4. Distribution during pressure changes of twelve specimens of *Schistomysis spiritus* in light from below.

RESULTS

Schistomysis spiritus

Normal specimens of this species were found to show a marked response to pressure changes, swimming upwards with increased pressure and downwards with decreased pressure both in darkness and in vertical light from above (Figs. 1, 2).

In horizontal light the animals also exhibited a pressure response, moving towards the light with increased pressure and away from the light when the pressure was decreased (Fig. 3). Movement was with the head foremost in both cases.

With the observation tank vertical and the light coming from below (Fig. 4), the mysids again swam upwards with increased pressure and downwards with decreased pressure, as they did in darkness or when illuminated from above. Considered in relation to the incidence of the light, however, these movements are the reverse of those shown both in overhead and in lateral illumination.

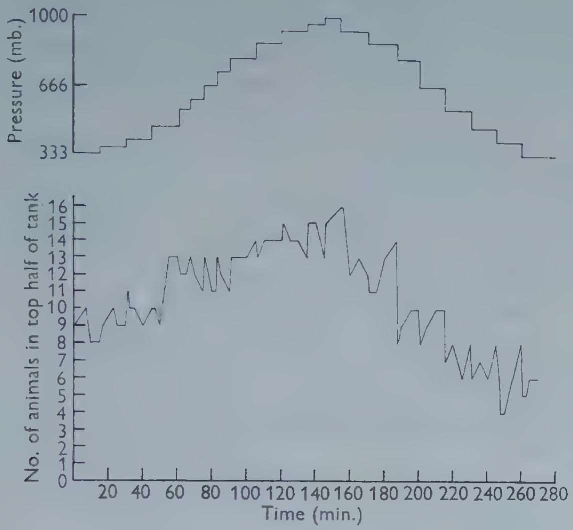

Fig. 5. Distribution of sixteen specimens of *Schistomysis spiritus* under constant overhead illumination, with cyclical change of pressure by frequent small increments and decrements.

The result of an experiment in overhead light in which the pressure was gradually increased and then decreased by frequent small changes is given in Fig. 5. A reproduction of the tidal pressure change was not attempted and the whole cycle in the experiment took only $4\frac{1}{2}$ hr., but the rate of change of pressure is of the same order as that which might be experienced in Port Erin bay due to the tides. It can be seen that responses were shown to each small pressure change and that there was also an over-all tendency for the animals to move upwards as the pressure increased and downwards as it decreased. The smallest changes of pressure in this experiment, at 15 and 30 min., were each equivalent to about 30 cm. of water and both produced definite responses. In another experiment responses were obtained to pressure changes as low as 15 mb. (i.e. 15 cm. of water).

The results given above refer to adult animals, but younger specimens were found to show the same responses to changes of pressure, and this was true even for those which had been released from the brood pouch only a few hours before.

Animals without statocysts. After removal of the statocysts the animals behaved in a similar manner to normal animals in light from above, moving upwards with increased pressure and downwards with decreased pressure (Fig. 6).

In darkness the same type of behaviour was again clearly shown (Fig. 7), and it was very noticeable that when the pressure was increased the animals tended to swim actively right at the surface of the tank, whereas after a decrease in pressure they were never observed in this position.

In horizontal light (Fig. 8) the result was again similar to that with normal animals. As noted by Foxon (1940) for *Hemimysis lamornae* (Couch), the movements towards or away from the light would sometimes be executed while the animal showed a dorsal light reflex all the time, i.e. with either the dorsal or ventral surface foremost. Quite often, however, the excursion would be made head first, with the dorsal light reflex only becoming apparent at the end of the movement. An interesting variant was also often seen in which the animal's first response to an increase of pressure would be to exhibit a dorsal light reflex facing upwards. It would then move slowly towards the light, dorsal surface foremost, and lean over backwards until it would be lying on its back facing the light source. Once in this position it would swim rapidly towards the light upside down or sometimes on its side. Having reached the end of the tank it would press its dorsal surface against the end plate and remain there until the pressure was decreased, when it would swim towards the other end of the tank with the ventral surface facing downwards.

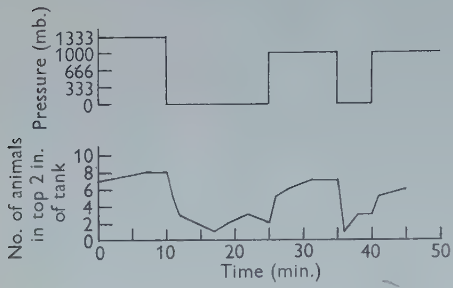

Fig. 6

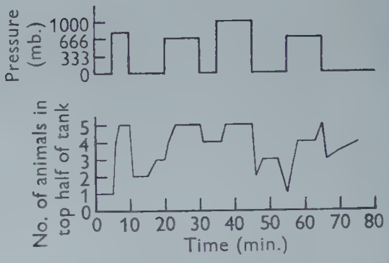

Fig. 7

Fig. 6. Distribution during pressure changes of fourteen specimens of *Schistomysis spiritus* without statocysts in overhead light.

Fig. 7. Distribution during pressure changes of five specimens of *Schistomysis spiritus* without statocysts in darkness.

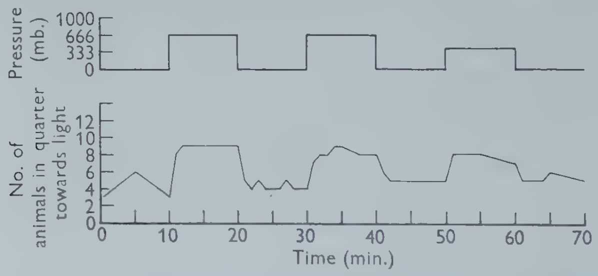

Fig. 8. Distribution during pressure changes of fourteen specimens of *Schistomysis spiritus* without statocysts in horizontal light.

In vertical light from below, the animals usually remained with their dorsal surfaces downwards and very close to the bottom, and increase or decrease of pressure rarely caused any change in this behaviour. Occasionally an animal was found swimming higher in the tank and in this case increased pressure almost invariably caused rapid swimming downwards towards the light, as is illustrated in the first part of Fig. 9, which is the track of a single individual. The interesting behaviour shown by this specimen in the later part of the same experiment was not seen in any other individual. After reaching the bottom in response to the initial increase in pressure, the animal remained there exhibiting the dorsal light reflex throughout the high-pressure period and the subsequent period of lowered pressure. Shortly after the second pressure increase, however, it made a succession of abortive attempts to leave the bottom. These attempts consisted of an upward movement of about 5 cm. executed head first, after which the animal turned over on to its back and swam obliquely to the bottom. Eventually, as recorded in the graph, it left the bottom and remained swimming for several minutes high up in the tank, orientated dorsal side up, and then, suddenly turning over, it exhibited a dorsal light reflex for a few seconds before swimming rapidly to the bottom. It remained on its back on the bottom throughout the following low-pressure period and more or less repeated the above procedure after the next pressure increase, but this time spent only a few seconds swimming high in the tank.

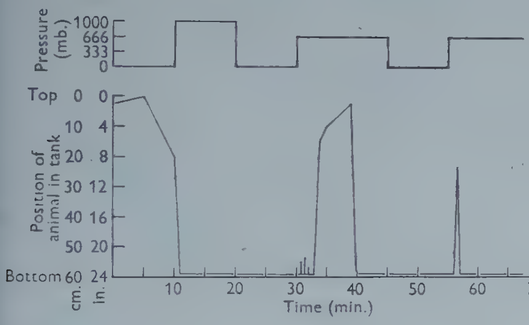

Fig. 9

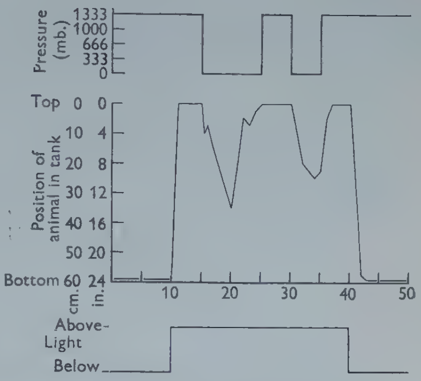

Fig. 10

Fig. 9. Track during pressure changes of a single specimen of *Schistomysis spiritus* without statocysts in light from below. The animal's abortive attempts to leave the bottom (see text) are indicated by short vertical lines.

Fig. 10. Track of a single specimen of *Schistomysis spiritus* without statocysts in an experiment in which the pressure and light direction were changed as indicated.

A further illustration of the importance of light as an orientating factor in animals lacking statocysts is furnished by Fig. 10, which shows the track of a single individual in an experiment during which the direction of the light was changed twice, in addition to the pressure changes. The animal maintained its dorsal surface towards the light throughout this experiment.

Siriella armata

In vertical light, either from above (Fig. 11) or below (Fig. 12), adults of this species behaved in a similar manner to *Schistomysis spiritus*, except that a smaller proportion of them moved upwards in response to increased pressure with the light from below.

In horizontal light the majority of the animals used made no migration at all, and only a few showed the same response as *S. spiritus*, swimming towards the source of light when the pressure was increased and away when it was decreased (Fig. 13). Under atmospheric pressure in horizontal light the animals remained more or less stationary on the bottom of the tank facing directly away from the light, and about half of them did not move from this position despite the pressure changes. However, about one-third of the specimens tested showed a change in their orientation when the pressure altered. After an increase of pressure they took up a position facing vertically upwards with their dorsal surfaces towards the light. Several animals maintained this orientation for as long as 30 min., until the pressure was again decreased, when they once more became horizontal, facing away from the light.

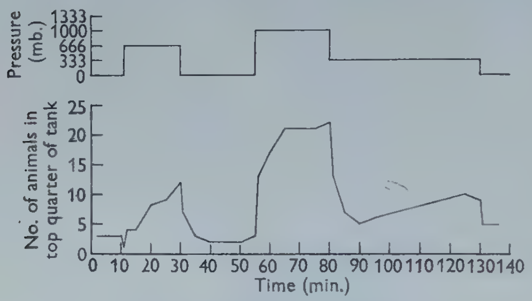

Fig. 11

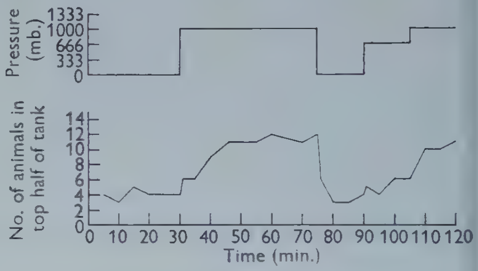

Fig. 12

Fig. 11. Distribution during pressure changes of twenty-four specimens of *Siriella armata* in overhead light.

Fig. 12. Distribution during pressure changes of twenty-four specimens of *Siriella armata* in light from below.

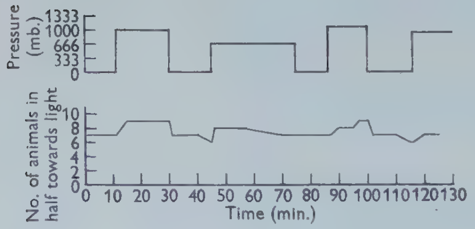

Fig. 13

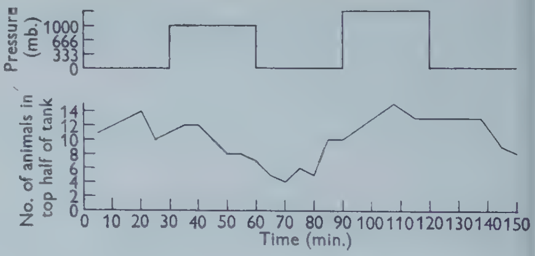

Fig. 14

Fig. 13. Distribution during pressure changes of fourteen specimens of *Siriella armata* in horizontal light.

Fig. 14. Distribution during pressure changes of sixteen specimens of *Siriella armata* under 16 mm. in length in darkness.

These results refer only to large individuals between 20 and 30 mm. in length. Smaller animals, less than about 15 mm. in length, gave very inconclusive results, as in the example illustrated in Fig. 14. This is markedly different from the situation in *S. spiritus*, in which all stages, including newly hatched specimens, reacted strongly to pressure changes.

Normal individuals of *Siriella armata* showed a greater tendency to cling to the surfaces of the observation tank than did *Schistomysis spiritus*, and when the statocysts were removed this tendency was even more marked; Foxon (1940) noted this effect of the removal of the statocysts in *Hemimysis lamornae*. In the present species it was found that if the animals were shaken free from the sides they exhibited a dorsal reflex only until they once more came into contact with a solid surface, when this clinging position was usually resumed. No obvious pressure response was obtained from these animals when the statocysts had been removed.

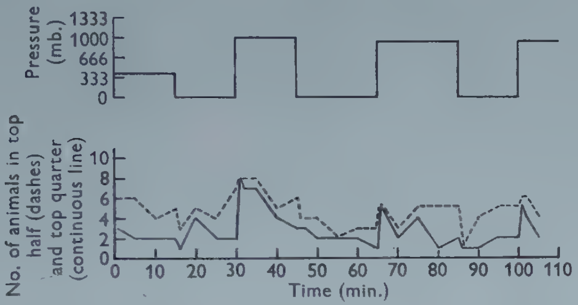

Fig. 15. Distribution during pressure changes of a mixed population of eight *Praunus neglectus* and six *P. flexuosus* in overhead light.

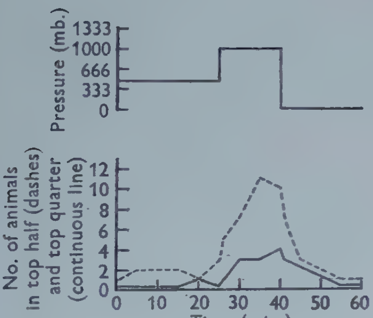

Fig. 16

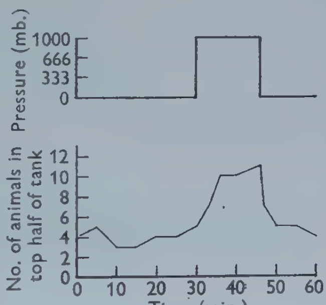

Fig. 17

Fig. 16. Distribution during pressure changes of a mixed population of twelve *Praunus neglectus* and two *P. flexuosus* in overhead light.

Fig. 17. Distribution during pressure changes of a mixed population of twelve *Praunus neglectus* and two *P. flexuosus* in light from below.

Praunus flexuosus *and* P. neglectus

These two species were grouped together because of the difficulty of separating them before they were used in the experiments. The numbers of each species were determined after the experiments, and are given in the legends to the figures.

Experiments with vertical light from above or below gave results essentially similar to those obtained with the other two species (Figs. 15–17), except that in some cases (e.g. Fig. 15) the initial migration at each pressure change was followed by a reverse migration; this tended to return the animals to their original positions in the tank. This is well shown by the record of numbers in the top quarter in Fig. 15, and to a lesser extent by that of the numbers in the top half. In the experiment recorded in Fig. 16 the result was more like those obtained with the other two species.

In horizontal light the pressure response was again orientated to the direction of the incident light, but the results were slightly different from those obtained with either *Schistomysis* or *Siriella*. Figs. 18 and 19 show that increased pressure induced movement towards the light source, but no movement away fromthe light was recorded in response to decreased pressure. The animals showed a marked tendency to orientate so that they were facing the light, and did not show the dorsal light reflex.

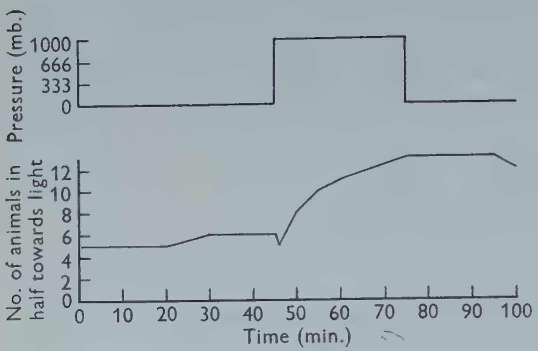

Fig. 18

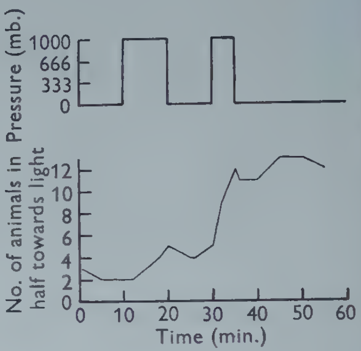

Fig. 19

Fig. 18. Distribution during pressure changes of a mixed population of twelve *Praunus neglectus* and two *P. flexuosus* in horizontal light.

Fig. 19. Distribution during pressure changes of a mixed population of twelve *Praunus neglectus* and two *P. flexuosus* in horizontal light.

A few experiments were conducted with these animals after the removal of the statocysts, and the same marked clinging tendency was shown as with *Siriella*. However, orientation to light was apparent even while the animals were clinging to a surface, since if they were illuminated laterally they would lean over so that the dorsal surface tended to be turned towards the light, and if they were facing the light the anterior end was lowered and the posterior end was raised so that again the dorsal surface was partly turned towards the light. Occasionally an increase of pressure produced a definite response in an animal, and this invariably consisted of swimming towards the light whether it involved upward, downward or horizontal movement.

DISCUSSION

Delage first demonstrated the balancing function of the statocysts in 1887 and since then various authors have been concerned with the problems of orientation and movement in mysids.

Von Buddenbrock (1914) did experiments with normal mysids and also with animals from which the statocysts had been removed; he came to the conclusion that mysids lacking statocysts orientate in the light by the dorsal light reflex, and in darkness by a general position reflex ('der allgemeine Lagereflex'). He suggested (pp. 506–7) that this latter orientating mechanism might result from the action of gravity on the internal organs suspended from the body walls by mesenteries, but the actual working of the reflex was, and still is, not understood.

Foxon (1940), working mainly on *Hemimysis lamornae*, confirmed the importance of orientation to gravity in normal mysids, and found that when the animals were dark-adapted the effect of light was to produce a positive geotaxis, presumably via the statocysts. The direction of the light was found to have no influence on the orientation of the response in normal animals, but once the statocysts had been removed the light direction did become important for orientation. Franz (1911) and Fraenkel (1931) had described the phototelotaxis of light-adapted *H. lamornae*, in which the animals swam alternately towards and away from the light source. Foxon (1940) stated that in his experience this behaviour only occurred in horizontal light, and that in vertical light from above the animals made horizontal excursions near the bottom.

Certain conclusions can be drawn from these observations. First, it seems that in normal mysids orientation to gravity is of major importance; vertical light apparently has little effect on the animals' orientation, but when it is directed horizontally it does become important in this respect. On the other hand, after removal of the statocysts the direction of incident light has more influence on the orientation than gravity, although in the absence of light, orientation to gravity is still possible.

In the present work, by observing the responses of the mysids to pressure which itself has no directional component, these conclusions have been further substantiated. The dominant effect of gravity over light in the orientation of normal mysids is shown by the fact that their pressure response is orientated to gravity even when they are illuminated from below. However, in *Siriella armata* with the light coming from below appreciably fewer animals moved upwards in response to increased pressure than did so in overhead light. This may indicate an influence of the light direction in this species at least.

In the experiments with horizontal light the orientating function of the light is well shown by the pressure responses of all the species tested, even in those cases in which no migration occurred.

Experiments in vertical light with animals lacking statocysts show that after the operation the importance of light for orientation is increased, but the behaviour exhibited by these animals in darkness (Fig. 7) shows that, even when the balancing organs have been removed, orientation to gravity is still possible (presumably via the general position reflex of von Buddenbrock).

Orientation of the body in mysids seems to be under the control of at least three different systems. The most important is the orientation to gravity by means of the statocysts. Secondly, there is the dorsal light reflex via the eyes, which in nature would normally reinforce the orientation to gravity. In the laboratory, by making the two factors operate in opposite directions, it can be shown that control by the statocysts is normally the stronger, and that the dorsal light reflex only becomes dominant when the statocysts are removed. The third mechanism is the general position reflex and this is obviously the least dominant of the three, since only in animals without statocysts and in total darkness does it become apparent.

Once it has been established that an animal responds to pressure changes in the laboratory, it becomes of interest to consider the possible significance of this behaviour in nature. For a planktonic animal a pressure response could obviously be useful in enabling it to detect, and to compensate for, any vertical displacements that might occur, and this could apply to mysids at night when many are found in the plankton. However, these animals spend most of their time close to the bottom, and in this position would not experience these vertical displacements, but they would be subjected to the slow pressure changes associated with the rise and fall of the tides. The experiments on *Schistomysis spiritus* with frequent small changes of pressure (Fig. 5) indicate that at any rate this species can respond to these slow changes.

Liao (1951), working on *Schistomysis spiritus* in Port Erin bay, studied the aggregation of the animals at the water's edge at different times of the day and at different states of the tide. He found that young forms were common at the water's edge near the time of high water, irrespective of the time of day, but only at night were large concentrations of older individuals found in this region. When low water occurred at night the population at the water's edge was very small. His results indicate that the animals have a tendency to move to the water's edge each night and with each rising tide, and the reverse tendency during the day and at low tide. Liao also noted that dull weather during the day increased the numbers found at the water's edge.

Elmhirst (1931, 1932) observed *Schistomysis spiritus* and *Praunus flexuosus* to move into the intertidal region with the incoming tide at night and he called these species 'tidal migrants' accordingly. He also observed that on nights with bright moonlight this shoreward migration did not occur, and this agrees with Liao's findings that light intensity is important in these movements.

Laboratory observations (Foxon, 1940, and personal observations) have confirmed that several species of shallow-water mysids tend to leave the bottom more readily in darkness or very low light intensities. It appears, therefore, that both pressure and light intensity are important factors in controlling the activity and distribution of these species. Laboratory observations that upward migration in these animals may be induced both by low light intensities and by increased pressures are completely in accord with field observations that the greatest numbers near the water's edge occur at night and near the time of high water.

SUMMARY

1. Four mysid species have been found to exhibit responses to changes in hydrostatic pressure.

2. The orientation of these responses has been observed in normal animals, and also in ones from which the statocysts had been removed. The results have helped to confirm earlier work on mysids, and indicate that at least three mechanisms are involved in orientation in this group. These mechanisms are (*a*) orientation to gravity via the statocysts, (*b*) the dorsal light reflex and (*c*) the general position reflex.

3. One of the species used has been shown to respond to slow pressure changes of the same order as those due to tidal rise and fall, and it is suggested that the pressure responses may be important in the tidal migrations of shallow-water mysids.

I am very grateful to Dr D. I. Williamson for much help and encouragement in this work and to Mr J. S. Colman for his critical reading of the manuscript. I would also like to thank Mr E. Sloane and Mr A. Sale for help in the construction of the apparatus.

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THE PHYSICAL PROPERTIES OF THE SWIMBLADDERS OF SOME SOUTH AMERICAN CYPRINIFORMES

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(*Received* 8 *December* 1960)

INTRODUCTION

It has recently been shown in experiments on eleven species of Cyprinidae and on the siluroid *Amiurus* (Alexander, 1959*a*) that the pressures of the gas in the swimbladders of these fish exceed the pressures in the surrounding water, at the depth to which they are adapted. No such excess internal pressure was found in any of seventeen species of fish from orders other than the Cypriniformes (Alexander, 1959*d*). It was suggested that the excess internal pressure is necessary for the operation of the Weberian mechanism which is characteristic of the Cypriniformes. The maintenance of this pressure requires a stronger swimbladder wall than is generally found outside the Cypriniformes.

On account of the excess internal pressure and of the finite extensibility of the swimbladder wall, the volume of the swimbladder of a cyprinoid changes less as the fish decreases or slightly increases its depth than would an unconstrained bubble of gas (or the swimbladder of a fish from another order) exposed to the same changes of external pressure. The greater the excess internal pressure and the lower the extensibility (and the extensibility falls with increasing excess internal pressure) the more marked will be this limitation of change of volume, and so of buoyancy, with depth (Alexander, 1959*a*, equation 5). Such limitation will be advantageous in swimming to fish whose densities approximate to that of the water in which they live, as do those of the Cyprinidae investigated (Alexander, 1959*b*), but disadvantageous in so far as it limits the sensitivity of the Weberian mechanism. It was found (Alexander, 1959*c*) that the anterior sac of the swimbladder, to which the Weberian ossicles are attached, is considerably more extensible than the posterior sac, whose function is solely hydrostatic.

The order Cypriniformes is divided (Berg, 1940) into four suborders, the Characimoidei, Gymnotoidei and Cyprinoidei, grouped as the division Cyprini, and the Siluroidei, isolated in the division Siluri. The work summarized above was carried out on Cyprinidae (suborder Cyprinoidei), apart from three experiments on a single species from the Siluroidei. It was clearly desirable to determine whether the properties of the swimbladder established for the Cyprinidae were general properties of the order.

The British Cypriniformes are all Cyprinoidei, but members of all three other suborders are abundant in tropical South America. I was therefore delighted to accept an invitation to join the Cambridge Expedition to British Guiana, 1960, on which I carried out the experiments on members of these suborders reported in this paper.

MATERIALS

The following spedies were used in this investigation.

Characinoidei:	*Serrasalmo rhombeus* (L.)
	Myloplus rubripinnis (Müller & Troschel)
	Brycon falcatus Müller & Troschel
	Creatochanes affinis Günther
	Anostomus plicatus Eigenmann
Gymnotoidei:	*Sternopygus macrurus* (Bloch & Schneider)
Cyprinoidei:	*Carassius carassius* (L.)
Siluroidei:	*Pseudopimelodus villosus* Eigenmann
	Trachycorystes obscurus (Günther)
	Trachycorystes galeatus (L.)

Apart from *Carassius* and *Sternopygus*, which were obtaned from dealers in Britain and Guiana, respectively, the fish were caught in the Moraballi River, British Guiana, and in the nearby parts of the River Essequibo, of which it is a tributary. Rods, a night line, and an eel trap were used.

METHOD

The method was essentially that used by Alexander (1959*a*), modified to make the apparatus as robust and compact as possible, and so suitable for use in camp. The glass flask of the original apparatus was replaced by the hollow Perspex cylinder shown in section in Fig. 1. The axis of this cylinder is vertical. The removable upper end is held in place by three brass bolts, symmetrically arranged, and the joint is sealed by a greased rubber washer. Two tubes pass through this end. The other, fixed, end is bored to take a small rubber bung through which a large steel pin passes. This pin is greased so that it slides easily up and down in the bung, whose flexibility allows its point to reach every part of the upper end of the cylinder. The cylinder was used in the vertical position in all experiments except J 24 and J25, when it was laid on its side.

The glass capillary of the original apparatus was replaced by a 1 m. length of natural polythene cannula tubing of reputed internal diameter 2 mm., which was fastened along a metre rule with Sellotape. The bore was determined more exactly by weighing the mercury which filled a measured length of this tube. No taper could be detected in the tube by measuring a column of mercury (about 30 cm. long) in different positions in it, and stretching of the tube under the pressures used was negligible compared to that of the Perspex cylinder, which was measured, found to be nearly constant, and corrected for. The cylinder expanded non-linearly by 0·10 ml. as the pressure in it was increased by 24 cm. Hg from atmospheric pressure.

The manometer and compressed air supply used in the original apparatus were replaced by a sphygmomanometer from which the armband and bag had been removed. The arrangement is shown in Fig. 2. Pressures could be produced with the sphygmomanometer's bulb, measured on its manometer, and released through the valve incorporated in the bulb. The head of water between the meniscus and the top of the cylinder was small enough to be ignored. No provision was made for reducing the pressure in the apparatus below that of the atmosphere.

The fish were kept in water 15 cm. or less deep for 2 hr. or more (J 21, 1 hr.) before the experiment and killed by pithing immediately before the experiment, except for *Serrasalmo* and *Myloplus*, which were caught some miles from the camp at depths up to 1½ m. and pithed immediately. The experiments on these species were started between 3 and 7 hr. after the death of the fish, but the fish were kept as cool as possible in the interval. In three cases (J 6, J 12 and J 13) the body cavity was opened and the pneumatic duct ligatured immediately after the fish were killed. It seems probable that all these fish adapted themselves to atmospheric pressure in the few moments between landing and death by the *Gasspuckreflex*, which takes place rapidly (see, for instance, Moehres, 1940). In all experiments it was assumed that the fish were adapted to life at zero depth.

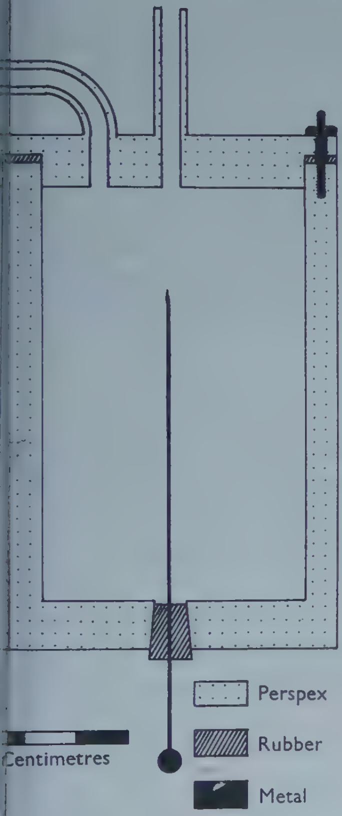

Fig. 1

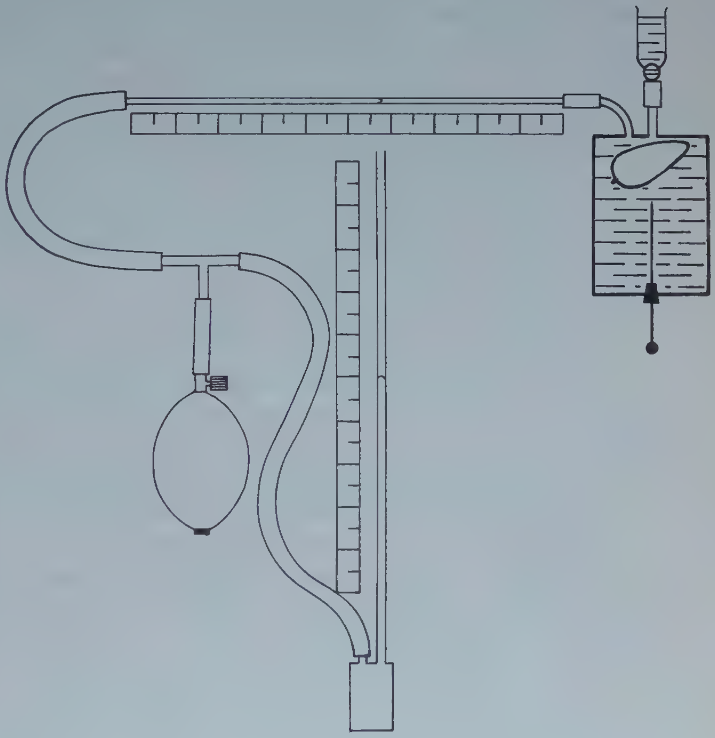

Fig. 2

Fig. 1. The Perspex cylinder in vertical section.

Fig. 2. The complete apparatus.

The fish were dried with a rag and weighed. No balance was available during the experiments on *Sternopygus*, but the specimens were preserved in 5 % formaldehyde and weighed at the earliest opportunity, 3 weeks later. A 40 g. specimen of *Gymnotus carapo*, weighed fresh and again after a month in 5 % formaldehyde with the body cavity open, showed no appreciable change in weight. It seems probable, therefore, that the weights given for *Sternopygus* approximate closely to the fresh weights.

The body cavity was opened and the pneumatic duct ligatured, and cut on the oesophageal side of the ligature. The swimbladder was then dissected out. Two ligatures were tied round the ductus communicans, which was then cut between them. The two sacs of the swimbladder, thus separated, were used separately in the experiments. In the Siluroidei the swimbladder consisted of a single sac which was so closely attached to the vertebral column that it was found convenient to leave a section of the vertebral column and associated muscles attached to it.

A swimbladder sac was placed in the Perspex cylinder, which was closed, bolted and filled with water from the funnel, great care being taken to exclude air bubbles. The tube was then attached and more water admitted from the funnel to give a meniscus at a convenient position in the tube. The pressure was then increased by stages to 24 cm. Hg above atmospheric pressure, and the position of the meniscus noted at each stage. This was equivalent to part I of the laboratory technique (Alexander, 1959*a*) and was repeated several times.

The swimbladder sac was then pierced by means of the needle, so that some of the gas escaped, and the swimbladder gas was thereafter always at the same pressure as the surrounding water. The pressure was again increased several times by stages to 24 cm. Hg, and readings were taken. This was equivalent to part II of the laboratory technique (Alexander, 1959*a*).

The calculations were made exactly as for the laboratory technique, and the results expressed in terms of the quantities defined by Alexander (1959*a*, p. 316).

Experiments J 15 (*Serrasalmo*) and J 23 (*Myloplus*) were carried out on swimbladders artificially inflated to an abnormally high excess pressure, to determine the extensibility at pressures above the excess internal pressure. Inflation was through a blunt no. 20 hypodermic needle tied as a cannula into the ductus communicans. A ligature was pulled tight round the duct as the needle was withdrawn.

The apparatus was suitable for swimbladder sacs having volumes between about 1·5 and 12 ml., and so, in general, for work on fish weighing between 50 and 200 g. In experiments on larger fish (J 2 and J 15) readings could not be taken over the full pressure range of 24 cm. Hg.

A few experiments, of which the results are given in Table 2, were made on groups of small, pithed fish which were put into the apparatus together intact after being kept for varying short periods in water 15 cm. deep. In these experiments the properties of the two swimbladder sacs could not, of course, be determined separately. Only part I of the experiment could be carried out, but the part II curve was calculated from Boyle's Law and the readings at 12 and 24 cm. Hg in part I. The part I and part II curves had never been found, in the experiments on isolated swimbladder sacs, to differ significantly over this range of pressures. Part II of the experiment on the anterior sac in J 11 had to be calculated in the same way after an accident with the apparatus. Experiments in which part II was calculated were not used for the calculation of extensibilities.

RESULTS

The results of the experiments, apart from determinations of extensibility, are given in Tables 1 and 2. The mean results for *Amiurus* previously published (Alexander, 1959*a*) are included for the sake of completeness.

Table 1. *The percentage volume, excess internal pressure and relative sensitivity of the isolated swimbladder sacs examined*

			Anterior sac			Posterior sac				
	Expt.	Wt. (g.)	Volume (%)	Pressure (cm. Hg)	Relative sensitivity	Volume (%)	Pressure (cm. Hg)	Relative sensitivity	Total % volume	Overall relative sensitivity
Characinoidei										
Serrasalmo rhombeus	J 2	282	5·9	1·7	0·59	1·4	1·3	0·29	7·3	0·53
	J 6	—	—	2·3	0·56	—	2·0	0·26	—	0·48
	J 10	186	—	—	—	1·5	1·2	0·43	—	—
	J 11	99	6·3	1·6	0·50	1·7	2·2	0·14	8·0	0·43
	J 12	106	5·3	1·9	0·58	1·8	1·1	0·69	7·1	0·61
	Mean		5·8	1·9	0·56	1·6	1·6	0·36	7·5	0·51
Myloplus rubripinnis	J 13	154	1·9	1·1	0·66	3·7	0·9	0·69	5·6	0·68
Brycon falcatus	J 20	199	1·9	1·4	0·78	5·7	2·9	0·36	7·6	0·48
Gymnotoidei										
Sternopygus macrurus	J 24	56	0·6*	—	—	5·2	4·6	0·13	5·8	—
	J 25	75	0·4*	—	—	4·6	4·3	0·06	5·0	—
	Mean		0·5	—	—	4·9	4·5	0·10	5·4	—
Siluroidei										
Pseudopimelodus villosus	J 19	64	5·9	0·5	0·89	—	—	—	5·9	0·89
Trachycorystes obscurus	J 17	32	4·5	1·0	0·86	—	—	—	4·5	0·86
	J 21	53	5·1	0·5	0·92	—	—	—	5·1	0·92
	Mean		4·8	0·8	0·89	—	—	—	4·8	0·89
Trachycorystes galeatus	J 8	139	3·4	0·6	0·90	—	—	—	3·4	0·90
Amiurus nebulosus†	Mean		5·8	0·6	0·87	—	—	—	5·8	0·87

* Estimated values. † From Alexander (1959*a*).

Table 2. *Results of the experiments with groups of intact fish*

	Expt.	No. of fish	Total wt. (g.)	Volume (%)	Pressure (cm. Hg.)	Relative sensitivity
Characinoidei						
Creatochanes affinis	J 3	3	37·0	5·6	1·5	0·68
	J 4	3	30·4	5·8	1·3	0·73
	J 5	3	40·8	6·9	1·7	0·65
	Mean			6·1	1·5	0·69
Anostomus plicatus	J 16	5	31·2	6·4	2·9	0·64

The Characinoidei examined constitute a rather diverse sample of the suborder. They were found to have excess internal pressures between about 1 and 3 cm. Hg, as compared to the 2–3 cm. Hg most usual in Cyprinidae (Alexander, 1959*a*). Marked differences in opposite senses are found between the pressures in the two sacs in J 12 and J 20, but in other cases the pressures are roughly equal. Evans & Damant (1928) found roughly equal pressures in the two sacs of the roach swimbladder. The mean total percentage volumes for the species examined lie between 5·6 and 7·5, as compared to 5·8 and 9·9 for Cyprinidae (Alexander, 1959*a*). In *Myloplus* and *Brycon* (and in the many species which were dissected but on which no measurements were made) the anterior sac is considerably smaller than the posterior, as in most Cyprinidae, but in *Serrasalmo*, where it is used in sound production, it is much larger.

Sternopygus was the only genus of Gymnotoidei examined. The anterior sacs were too small for investigation in the apparatus, but their approximate volumes were calculated from their linear measurements. The posterior sac was found to have an excess internal pressure of about 4·5 cm. Hg in each case; though this is high, it is below the mean values found for roach, rudd and bream (Alexander, 1959*a*). The relative sensitivities of the posterior sacs were correspondingly low, and as the anterior sacs were so small the overall relative sensitivities of the swimbladders must have been very low; even if the relative sensitivity of each anterior sac was nearly 1 (and 0·5 is a much more probable figure) the mean overall sensitivity cannot have exceeded 0·18. No other fish has so far been shown to have so low a relative sensitivity. It seems likely that a low relative sensitivity, and therefore a low rate of change of buoyancy with depth, would be particularly valuable to a fish which is not a powerful swimmer. Lissmann (1958) considers that the peculiar and (we must suppose) weak swimming mechanism of the Gymnotoidei is essential to the functioning of the electric sensory system. Relatively small anterior sacs are common in Gymnotoidei (see the illustrations by Ellis (1913) of the swimbladders of *Gymnotus*, *Sternopygus* and *Eigenmannia*; I have also observed that the anterior sac is much smaller than the posterior in *Electrophorus*). It therefore seems likely that low overall relative sensitivity is widespread in the suborder; it is unfortunate that experiments could only be carried out on one species.

The Siluroidei which have been examined are all Bagridae. They all have rather small swimbladders with excess internal pressures between 0·4 (*Amiurus*) and 1 cm. Hg. These pressures are strikingly below those found in the Cyprini. The relative sensitivity is consequently high (about 0·9); the swimbladder wall thus exercises very little restraint on changes of buoyancy with depth. The apparatus was unsuitable for the investigation of the swimbladder of any of the numerous Siluroidei in which it is markedly reduced.

Figs. 3 and 4 are graphs of extensibility against excess internal pressure for anterior and posterior sacs, respectively. Curves for *Carassius* as well as Guianese fish are included. These curves show that the extensibilities of anterior and of posterior sacs do not vary much between the Cyprini examined, except that the enlarged sound-producing anterior sac of *Serrasalmo* is unusually inextensible. Anterior sacs are considerably more extensible than posterior sacs (see Alexander, 1959*c*).

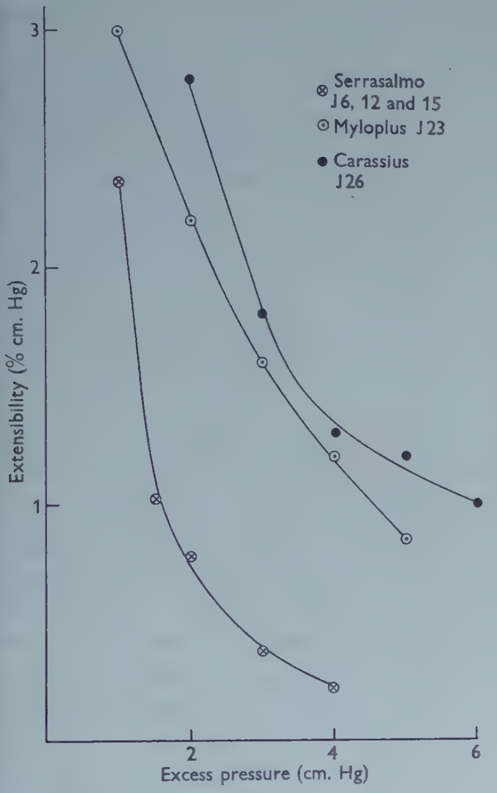

Fig. 3

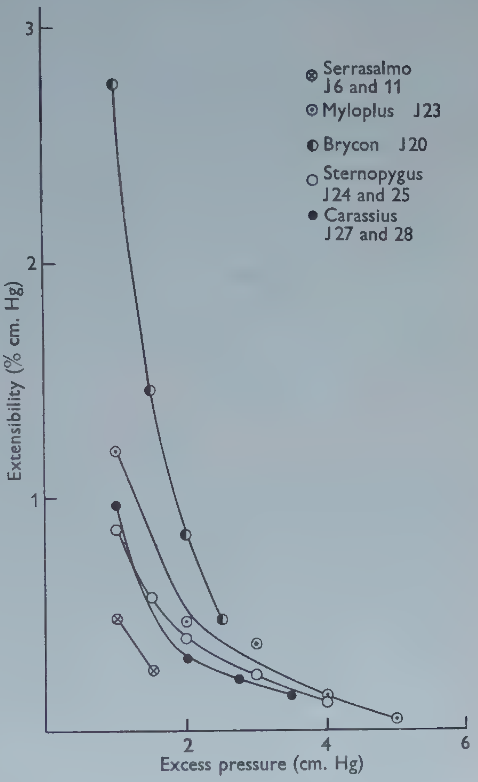

Fig. 4

Fig. 3. Extensibility of anterior sacs plotted against excess pressure.

Fig. 4. Extensibility of posterior sacs plotted against excess pressure.

The excess internal pressures of the Siluroidei were too low for it to be possible to obtain useful extensibility curves from the results of the ordinary experiments. Attempts were made to inflate siluroid swimbladders to higher excess pressures, but these failed owing to the weakness of the junction of the pneumatic duct with the swimbladder.

SUMMARY

The physical properties of the swimbladders of a variety of Characinoidei, Gymnotoidei and Siluroidei were investigated and found to be essentially similar to those previously reported for Cyprinidae. The Siluroidei, however, which were all Bagridae, had much lower excess internal pressures than the other Cypriniformes.

I am most grateful to the other members of the Cambridge Expedition to British Guiana, 1960, for their invitation to join them and for their help, especially in catching fish. The expedition was made possible by the generosity of numerous bodies and firms who made grants to it of money, equipment and provisions. There is space here only to mention the Colonial Office, the Godman Exploration Fund, Shell Tankers Ltd., Booker Bros. McConnell Ltd., the Calouste Gulbenkian Foundation (Lisbon), the Worts Travelling Scholars' Fund, the Pilgrim Trust and the University College of North Wales. The others, with these, are listed and thanked in the expedition's non-technical report. I wish also to thank Dr D. E. Christie of Bangor, who lent me the sphygmomanometer, Dr E. Trewavas of the British Museum (Natural History) who identified two of the catfish and Mr Joseph Jardim, of McDoom Village, East Bank Demerara, who gave me the *Sternopygus* and allowed me to work on them at his aquarium. We found kindness and help wheverer we went in British Guiana, especially from the staff of the Forest Office at Bartica, of the Georgetown Museum, and of British Guiana Timbers, Ltd., whose forest base, Wineperu, was a few miles from our camp.

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OSMOTIC AND IONIC CHANGES IN BLOOD AND MUSCLE OF MIGRATING SALMONIDS

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INTRODUCTION

Osmoregulation in teleost fishes, whether they live in fresh water or in the sea, is a physiological activity very closely related to their survival. Yet in spite of the importance of osmoregulation surprisingly little is known about how fish deal with the physiological problem inherent in living in hypoosmotic and hyperosmotic environments. The ability of some fishes to regulate in both environments during migrations is of even greater interest. In his classical review of osmoregulation in aquatic animals, Krogh (1939) emphasizes the lack of direct evidence in the study of migrating diadromous fishes. He says: '...A small number of teleosts are able to stand a fairly rapid transference from fresh water to sea water and vice versa, and some of these undertake regular migrations between the two media, but the peculiarities which are responsible for this power are not at all clear in spite of the considerable amount of work spent upon the problem.' Some advances can be recorded; thus Pyefinch (1955) in his review of the literature on the Atlantic salmon writes, '...the physiological and biochemical studies over the last twenty years or so have shown the advances that can be made now that general knowledge of endocrine organs and hormones has made it possible to investigate their function in fishes. Studies of the internal environment will not...provide the complete answer to the many outstanding problems of migration and smolt development, but further advances in this field, supplemented by appropriate investigation in the external environment, could provide a much more complete picture of these phases in the life-history of the salmon.'

In the years between the publication of these two accounts many data have been accumulated in the field of the physiology of migrating fishes, but relatively little information is available about the chemistry of the internal environment, about permeability, or about renal physiology, all of which would seem to be particularly relevant to a study of migration in diadromous fishes. The present study has been undertaken to provide information about the level of some ions in blood and muscle during the life-history and migratory periods of the salmon.

Such a migratory fish as the salmon might seem to be an ideal example for a study of euryhaline behaviour. In many ways this is so, but there are disadvantages. There is a long period in the life of the Atlantic salmon which is almost unknown, and when it is not possible to obtain any physiological material, namely the sea-water period. The adult fish almost always have to be studied in field conditions since they are large for the resources of most laboratory aquarium facilities, and for the same reason difficult to move from one place to another. Also, since they are economically important, the young stages are valuable as potential adults, and the adult ones valuable in themselves, so that the numbers required for physiological work are not always easy to obtain.

METHODS

Fish of the species *Salmo salar* (L.) were used for most of the analyses; wild fish were collected from many different places in Great Britain, and some young fish were reared from eggs in laboratory conditions. Comparative analyses were made also on other *Salmo* species in Britain, i.e. *S. trutta* (L.) as freshwater 'brown trout' or marine 'sea trout' and *S. gairdnerii* (Richardson), the rainbow trout (hatchery reared). Blood samples were obtained by cardiac puncture, with or without anaesthesia, without harming the fish. The most convenient anaesthetic was found to be 'Metacaine'* in a concentration of 1:10,000. Some other anaesthetics, viz. metycaine, trichlorethylene, carbon dioxide and urethane were tried but found to be less successful. Pre-spawning adult fish were netted at the mouths of rivers and blood was obtained from these by cardiac puncture immediately after the fish had been stunned.

Blood samples from very small fish were collected in soda glass cannulae, and immediately transferred to liquid paraffin in polythene sample tubes in the field. In the laboratory the blood samples were transferred to 'Damarda'†-lined watch-glasses which induced serum and a clot to separate. For larger fish, glass or nylon hypodermic syringes were used, and the blood was stored for short periods under paraffin in polythene bottles, and frozen with 'Drikold'. As soon as possible the blood was centrifuged, and the plasma separated.

The freezing-point depression of the blood (Δ, °C.) was measured in very small quantities (0·001 ml. or less) in silica capillaries, using Ramsay's cryoscopic apparatus (Ramsay, 1949; Ramsay & Brown, 1955). Sodium, potassium and calcium were estimated by flame photometry (EEL photometer) using standard solutions and dilutions of a standard sea water for comparison and calibration. Carbonate was measured in freshly collected blood samples by the micro-diffusion method of Conway (Conway, 1957). Chloride was estimated either by a Conway micro-diffusion method, and/or by a potentiometric titration with silver nitrate.

Some attempts were made to estimate trimethylamine and its oxide, either after a steam distillation extraction or by a microdiffusion method. Although considerable amounts were found in muscle extracts, none could be detected in blood samples of either the freshwater or marine salmon.

BLOOD SALT CONCENTRATIONS

(*a*) *During the life history*

This has been measured in blood from the young salmon embryos, in hatched alevins, fry, parr, smolts before and during their migration, in marine adults returning to spawn, in spawning adults and in the spawned kelts in both fresh water and sea water. The results are shown in Table 1 and in Fig. 1.

It can be seen that Δ_{blood} is at a constant level during the freshwater juvenile life, except for a rise during the first few weeks of life from 0·49° C. at hatching to 0·56° C.

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* M.S. 222 from Sandoz Ltd.

† 'Damarda' formite resin from Bakelite Ltd.

in the alevins and fry. This rise in blood concentration is apparent in the yolk-sac bearing alevin before feeding is initiated, and leads one to suppose that ionic regulation has begun already at this early stage. Busnel, Drilhon & Raffy (1946) measured the blood concentration in hatching eggs and found similar low figures for Δ_{blood} (0·49° C.) and a rise as the alevin develops (to 0·59° C. after 31 days). A rather higher figure (0·61° C.) is reported for 'Vesicular alevins' by Auvergnat & Sécondat (1941). The blood concentration rises slightly during the first year of freshwater life, but thereafter remains constant through the 'parr-smolt' transformation when the seaward migration begins.

When the parr metamorphoses into the smolt there is no significant change recorded for Δ_{blood}; that is, there is no increase in total blood concentration prior to entry into

Table 1. *Freezing-point depression* (Δ, ° C) *of blood of salmon*, Salmo salar

Stage in life history	Sampling time	Environment	Δ, °C. ± S.E. (*N*)
Eggs	January	Fresh water	0·49 ± 0·03 (12)
Alevins 6 days post-hatch	February	Fresh water	0·56 ± 0·02 (4)
Fry 3 months	April	Fresh water	0·56 ± 0·03 (32)
Parr 1 year 3 months	May	Fresh water	0·61 ± 0·02 (13)
Smolts 2 years 3 months	May	Fresh water	0·58 ± 0·08 (12)
Smolts 3 years 6 months	August	Fresh water	0·65 ± 0·10 (15)
Adult pre-spawning	July	Sea water	0·64 ± 0·01 (7)
Adult spawning	December	Fresh water	0·61 ± 0·05 (17)
Adult kelts	January	Fresh water	0·63 ± 0·01 (4)
Adult kelts	February	Sea water	0·69 ± 0·04 (7)

S.E. is standard error of mean; *N* is number of measurements.

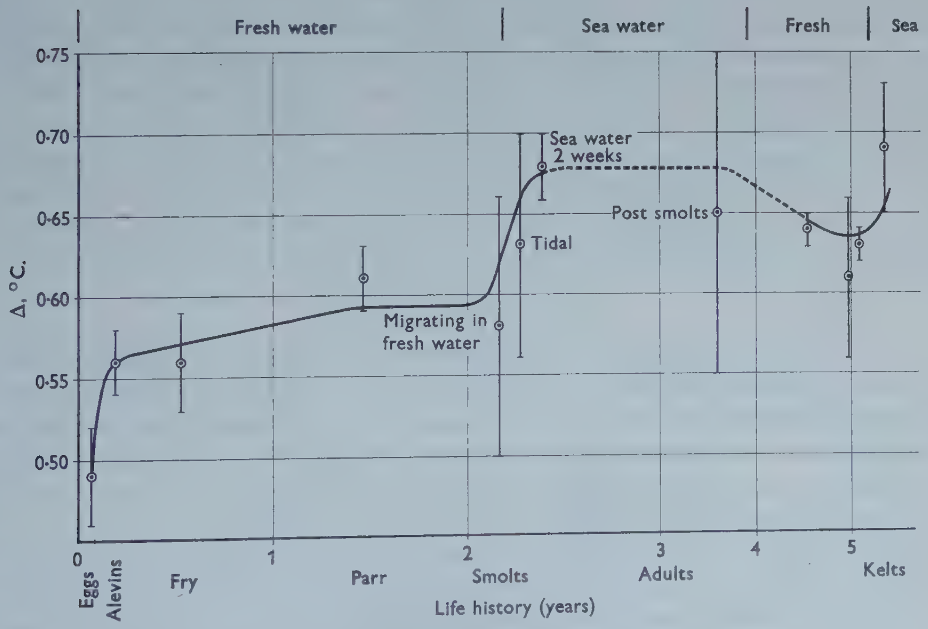

Fig. 1. Osmotic pressure of the blood of salmon at different times in its life history and in fresh water and sea water.
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sea water, nor is there evidence for a 'demineralization' of the blood to stimulate a sea-going urge (cf. Fontaine & Callamand, 1940, 1948; Kubo, 1953). However, a greater variability in the results was found at this stage and this may represent a degree of sensitivity of the smolt (Fontaine & Callamand, 1948), or of hormone 'unbalance' in the fish at this time (Hoar, 1951). A similar variability (in sodium content of the blood) has been reported by Koch & Evans (1959) for wild migratory smolts.

If smolts were kept in fresh water beyond the time of migration, Δ_{blood} was found to be higher than in the migrating smolts. For example, fish from the River Piddle at Wareham (Dorset) kept in tanks of running hard water at Stevenage (Water Pollution Research Laboratory) for 12 months, and hatchery-reared fish of similar age from the Scottish Hydro-Electric Board's hatchery at Invergarry, Inverness-shire, kept for a similar period in soft water, both showed this trend. There was no indication of a seasonal return to normal during the annual smolt migration; the rise observed could have been the result of ageing or of artificial feeding.

Very little is known of the life of the salmon in sea water and no material of this stage was obtained for the present study.

Adults from sea water, returning to the spawning grounds in the river, were captured in sea water at the mouth of the River Dee, Aberdeen. They had Δ_{blood} of the same order of that of other marine fishes, although it is about 10% lower in concentration than that of many estimates reported in the literature (Black, 1957). This lower value is probably due to the difference in techniques for measuring Δ_{blood}. There is no reason to believe that the figures obtained from these fish did not represent the level of blood concentration in sea-living salmon. When the adult salmon reached the spawning ground and had been in a freshwater environment for 3–4 months, Δ_{blood} dropped to 0·61° C., which was a little higher than the level for juvenile salmon in fresh water. Spawned fish, although starving and exhausted, are still able to regulate since the blood does not fall below this level of concentration. However, ability to osmoregulate is impaired to some degree, as the spawned kelts after 5–6 months of starving freshwater life cannot survive or maintain their normal blood concentration when transferred directly to sea water. Similar values are reported in the literature for adult salmon; Benditt, Morrison & Irving (1941) give $\Delta_{\text{blood}} = 0{\cdot}77°$ C. for salmon in $\frac{1}{2}$ sea water ($\Delta = 0{\cdot}87°$ C.), and figures reported for adult spawning salmon in fresh water are $\Delta_{\text{blood}} = 0{\cdot}64$–$0{\cdot}66°$ C. (Fontaine, 1954; Hoar, 1953).

(*b*) *Other salmonid species*

Freezing-point measurements of the blood of two other *Salmo* species obtainable in Britain are shown in Table 2. No significant differences could be found between fish of the different species of similar age groups. The same general rise in blood concentration, observed in *S. salar* with increasing age, can be seen in *S. trutta* and *S. gairdnerii*.

Some figures for the Pacific genus *Oncorhynchus* from the literature seem to be considerably higher in range. Thus, Greene (1926) gives $\Delta_{\text{blood}} = 0{\cdot}61$–$0{\cdot}67°$ C. for adults of *O. tchawytscha* in fresh water and 0·76° C. for these fish in sea water; and Kubo (1953), for *O. masou* gives 0·60–0·69° C. for fish in fresh water and 0·75–0·90° C. for fish in sea water. It is possible that this difference is a real one, related to the difference in species or environment, but it seems more probable that it reflects

Table 2. *Freezing-point depression of blood* (Δ, ° C.) *of other salmonid species*

Species	Stage in life history	Environment	Δ, °C. ± s.e. (*N*)
S. trutta	Eggs 6 days	Fresh water	0·47 ± 0·03 (24)
	Parr 8 cm.	Fresh water	0·57 ± 0·06 (13)
	'Smolt' 14 cm.	Fresh water	0·57 ± 0·01 (13)
	Adult 20 cm.	Fresh water	0·59 ± 0·01 (7)
	Finnock 20 cm.	Sea water	0·69 ± 0·27 (7)
S. gairdnerii	Parr 9 cm.	Fresh water	0·57 ± 0·03 (7)
	'Smolt' 15 cm.	Fresh water	0·55 ± 0·07 (23)
	Adult 25 cm.	Fresh water	0·57 ± 0·02 (23)
	Adult 25 cm.	Sea water (6 days)	0·71 ± 0·02 (4)

s.e. is standard error of mean; *N* is number of measurements.

Table 3. *Freezing-point depression of blood of salmonids (from the literature)*

Species	Stage in life history	Environment	Δ_{blood}, °C.	Author
S. salar	Eggs hatching	Fresh water	0·49	Busnel *et al.* (1946)
	Alevins 31 days post-hatch	Fresh water	0·59	Busnel *et al.* (1946)
	'Vesicular alevins'	Freshwater	0·61	Auvergnat & Sécondat (1941)
	Adult	½ sea water (Δ = 0·87° C.)	0·77	Benditt *et al.* (1941)
	Adult	Fresh water	0·64	Benditt *et al.* (1941)
	Adult	Fresh water	0·65	Fontaine (1954); Hoar (1953)
	Adult spawning	Fresh water	0·66	Fontaine (1954)
S. gairdnerii	Adult	Fresh water (Δ = 0·02° C.)	0·50	Busnel (1943)
O. tchawytscha	Adult	Sea water	0·76	Greene (1926)
	Adult	Fresh water	0·74	
	Adult spawning	Fresh water	0·67	
	Adult spawning	Fresh water	0·61	
O. masou	Smolt, migrating	Fresh water	0·70–0·76	Kubo (1953, 1955)
	Adult	Fresh water	0·60–0·69	
	Adult	Sea water	0·75–0·90	

different techniques of freezing-point measurement. A summary of the figures reported in the literature for different species is given in Table 3.

(*c*) *Transfer to different salinities*

The osmotic changes in the blood after transfer of the fish from fresh water to sea water is of particular interest in these migrating fish. The experimental transfer, to sea water and its dilutions, of young parr and smolts (1 and 2 years old) of three species of *Salmo* has been described in previous papers (Parry, 1958, 1960). In general, salmonids of age group 2+ years were able to tolerate a direct transfer from fresh water to full strength sea water. The changes in blood concentration following the transfer were measured by determining the freezing-point depression. It was found that for fish of the 2+ year age group 150–300 hr. were required for the fish to be able to regulate its blood concentration to the normal levels, except in the case of the migrating smolt of *S. salar* which regulated its blood within a 24 hr. period.

An attempt was made to find out what happened to smolts during similar conditions in a river. Smolts were trapped at a station (on the River Coquet, Northumberland) just above tidal water, and were transferred a mile downstream into tidal water. The blood concentration of these fish can be compared with that of fish living naturally in this environment, and also with fish from the same population transferred to running sea water in the laboratory. The temperature of the river water and of the running sea-water tanks was about 10° C. during these experiments (Table 4). Freezing-point messurements of the water in the tidal reach of the river gave values of Δ between 0·09 and 1·00° C. at different states of the tide. A few fish were caught (by rod and line) in the same part of the river and the blood was sampled; the experimental fish were held in a keep-box immersed in the river. It is interesting to note that the naturally occurring fish, which had been in the tidal reaches for an unknown time, had blood no more concentrated than that of fish in fresh water above the tide. Of the smolts confined in a keep-box, the ones sampled after three tides showed a higher blood concentration than those sampled after seven tides. Two explanations can be put forward for this: (1) that the abrupt transfer to a higher salinity could not be compensated by the osmoregulatory mechanisms; or (2) that diuretic loss of water consequent upon the confinement in the keep-box was preventing the reduction in urine flow which is a necessary part of the physiological adjustment of the fish to higher salinities.

Table 4. *Transference of migrating salmon smolts to sea water and tidal water*

(Temperature about 10° C.)

Conditions	Δ_{blood}, °C ± S.E. (N)
Smolts from freshwater trap	0·59 ± 0·05 (12)
Smolts naturally in tidal part of river	0·60 ± 0·02 (4)
Smolts kept in tidal reaches for 3 tides	0·67 ± 0·05 (6)
Smolts kept in tidal reaches for 7 tides	0·63 ± 0·07 (3)
Smolts in sea water 1 day	0·65 (2)
Smolts in sea water 3 days	0·73 (2)
Smolts in sea water 12 days	0·68 ± 0·02 (8)

S.E. is standard error of the mean; N is number of measurements.

Fish from the same population, transferred directly to running sea water, also showed a rise in blood concentration above the level normal for these fish in sea water, but this was controlled within 12 days.

A similar transfer of adult fish, but from fresh water to sea water, was attempted in January 1959 (Table 5). The design of this experiment was the same as the previous one, i.e. the fish were transported from the freshwater traps and confined in keep-boxes immersed in sea water. The sea water in Morecambe Bay where the experiment was made, had a freezing-point depression of $\Delta = 1{\cdot}60°$ C. at the time of the experiment. The inability of the salmon kelts to osmoregulate is very much more marked than that of the salmon smolts in spite of a more favourable surface/volume ratio. Again, some of the increase in blood concentration could have been brought about by diuresis, but it is clear that whatever the cause, control had not been gained after 4 days in sea water. A contributory cause towards this inability to regulate could have been the low temperature of the water, which was at about 0° C. at this time, with ice forming on the inshore surface of the sea. A subsequent experiment in January 1960 with

Table 5. *Transference of salmon kelts to sea water*

(Temperature about 0° C.)

Conditions	Δ_{blood}, °C. ± S.E. (*N*)
Kelts trapped in fresh water	0·62 ± 0·07 (3)
Kelts 1 day in sea water	1·18 (2)
Kelts 2 days in sea water	1·02 (2)
Kelts 3 days in sea water	0·90 ± 0·02 (4)
Kelts 4 days in sea water	1·10 (1)

S.E. is standard error of the mean; *N* is the number of measurements.

kelts kept in larger tanks of running sea water indicated a better degree of control with Δ_{blood} maintained at about 0·90–1·00° C., but in this case the sea water in Morecambe Bay was much diluted by rain water run-off, and the freezing-point of the sea water was only $\Delta = 1{\cdot}20°$ C. The temperature in the 1960 experiment was similar to that of the earlier one.

A similar experiment with fresh-run adult fish moving into fresh water from sea water would be interesting, but so far is not available. The changes in the blood of a species of Pacific salmon, *Oncorhynchus nerka*, have been followed in natural conditions along the length of the Fraser River (Idler & Tsuyuki, 1958), and showed a gradual decline of blood concentration of about 5% of the marine level.

LEVELS OF INORGANIC IONS IN THE BLOOD

Estimates of the total ions in the blood can be calculated from the measurement of blood from the relationship that 283 m-equiv. monovalent strong electrolyte per kg. water has $\Delta = 1°$ C. (Ramsay & Brown, 1955). The freezing-point measurements of blood discussed in the previous section thus show that the total ionic concentration rises after hatching from 148 m-equiv./kg. to about 160–170 m-equiv./kg. There is no rise in premigratory smolts, but there is a rise of about 12% to 200 m-equiv./kg. in smolts in tidal water or in sea water; this is maintained in the pre-spawning marine adult. The ionic concentration of the blood drops a little while the adult completes its spawning migration into fresh water.

Results of analyses are shown in Table 6 and summarized in Fig. 2. Sodium and chloride are numerically the most important ions of the total ionic concentration. In blood from juvenile fish in fresh water, sodium and chloride are present in sufficient concentrations to account for almost all the ions present. But in adult pre-spawning fish the chloride concentration is considerably less than the value expected from measurements of the freezing-point. This anionic deficiency is not covered by high carbonate. Analyses of carbonate show very similar levels in both sea-water and fresh-water fish (within the limits of experimental error, which are quite serious with regard to this ion). It might be thought that sea-water levels of carbonate would rise with low plasma chloride, or drop with high plasma chloride (Fontaine & Boucher-Firly's hypothesis, 1934). The order of concentration of carbonate found, however, makes it unlikely that this ion can play much part in maintaining the total anionic concentration.

Both anionic and cationic organic constituents may be important, but analyses of plasma for trimethylamine oxide failed to indicate this substance in the plasma, although the methods employed should have been adequate (for the 1 ml. samples)

Table 6. *Ions in blood plasma of* Salmo salar

Fish, age, etc.	Medium	Ions, mM./kg. weight ± S.E. (*N*)					Total ions calculated from Δ m-equiv./kg.
		Na^+	K^+	Ca^{2+}	Cl^-	CO_3^-	
Parr	Fresh water	117·0 ± 17·6 (9)	2·19 ± 0·85 (8)	2·33 (3)	129·8 ± 21·5 (9)	—	160
Smolts	Fresh water	131·2 ± 52·0 (15)	3·03 ± 0·91 (11)	2·00 ± 1·40 (4)	182·7 ± 10·5 (3)	7·3 ± 3·0 (6)	174
Post-smolts	Fresh water	155·8 ± 44·0 (20)	3·28 ± 0·51 (19)	—	132·8 ± 45·3 (11)	—	185
Smolts	Sea water 2 weeks	159·3 ± 25·3 (11)	3·62 ± 1·63 (9)	—	166·3 ± 56·7 (4)	—	194
Adults	Sea water	211·9 ± 106·2 (19)	3·15 ± 2·05 (18)	3·43 ± 1·39 (13)	156·7 ± 72·2 (10)	11·0 ± 2·4 (11)	182
Adults	Fresh water (head of tide)	180·6 ± 76·9 (5)	1·86 ± 1·20 (5)	3·98 ± 1·09 (5)	—	—	—
Adults spawning	Fresh water	176·0 ± 37·0 (39)	3·03 ± 0·92 (29)	3·45 ± 0·90 (27)	172·4 ± 75·8 (12)	—	174
Adults	Fresh water	157·0 ± 62·0 (8)	3·77 ± 0·95 (7)	3·72 ± ·098 (6)	—	—	180
Adults kelts	Fresh water + 2–5 weeks in $\frac{2}{3}$ sea water	211·3 ± 15·5 (4)	4·08 ± 0·86 (4)	3·38 ± 1·33 (4)	—	—	200
Adult kelts	Fresh water + 2 days sea water	294·0 ± 72·5 (7)	3·44 ± 0·40 (5)	3·98 ± 1·30 (6)	—	—	—

S.E. is standard error of the mean; *N* is number of measurements.

if it had been present in significant quantities. No estimates of ammonia have been made. Further work is in progress to determine whether amino acids play an important part in osmoregulation during the transference from one salinity to another, as they do in some invertebrates (Shaw, 1958).

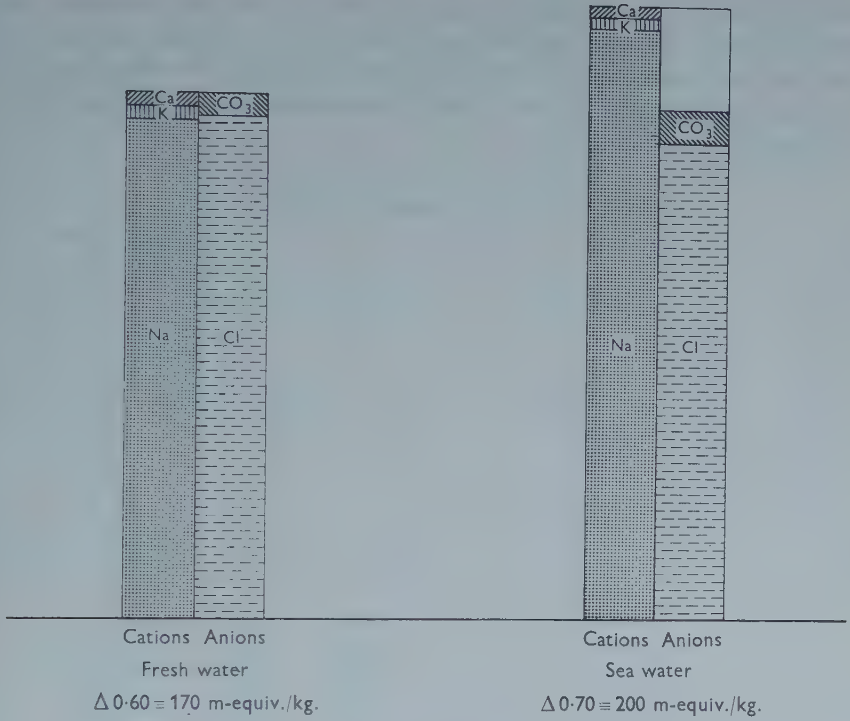

Fig. 2. The inorganic composition of the blood plasma of adult salmon in fresh water and sea water.

In general, the levels of individual ions follow the pattern expected from the freezing-point data. There is some indication that a size-concentration effect (Houston, 1959) is present; i.e. the concentrations of sodium and chloride in the plasma drop between that of the very young fingerlings and that of the 2-year smolt stage. Fresh-water and sea-water levels of plasma potassium seem to be about the same, and the range is well within that reported in the literature (Field, Elvehjem & Juday, 1943; Phillips & Brockway, 1958; Gordon, 1959*a*, *b*; Houston, 1959).

The plasma ion-levels of migrating smolts, and of smolts transferred artificially to sea-water, are interesting. Pre-migratory smolts in fresh water showed relatively variable figures for both freezing-point determinations and for inorganic analyses. This is shown by the larger standard errors of the figures in Tables 6 and 4. A similar variability is reported in plasma sodium levels of Atlantic salmon smolts (Koch & Evans, 1959). This high level of variability, however, is likely to be a reflexion of the curious physiological state of the smolt, rather than of direct osmotic significance.

ION CONCENTRATIONS IN MUSCLE

The levels of inorganic ions in the muscle are of interest, from the osmotic point of view, in relation to their levels in the blood. In a migratory fish such as the salmon even greater interest hinges on the response of the muscle, as well as the blood, in conformity with the external salinity changes which the animal encounters during its migrations. There are two possible responses of muscle tissue to such changes: (1) it can follow the osmotic and ionic changes in the blood, more or less closely, or (2) it can behave differently and thus act as a reservoir of ions or water to buffer blood changes (Drilhon & Pora, 1936).

Table 7. *Inorganic ions in salmon muscle*

			mM/kg. water			
Fish	Conditions	‰ water	Na	K	Ca	Cl
Smolt	Fresh water	—	16·3*	210*	3·14*	2·60*
Smolts	2 weeks in sea water	792	33·3	185	3·55	—
Adults	Sea water	676	28·1	206	2·84	6·12
Adults	Fresh water, head of the tide	641	29·7	194	2·95	—
Adults	Fresh water, spawning	776	66·4	261	2·84	—
Adults	Fresh water, kelts	805	45·5	199	2·22	—
Adults	Two-thirds sea water, 4 weeks	778	54·7	200	2·16	—
Adults	Sea water, 3 hours	779	80·7	205	2·99	—
Adults	Sea water, 2 days	822	36·7	238	1·94	—

* Figures for mM./kg. wet weight of muscle. All the figures are the means of a number of observations.

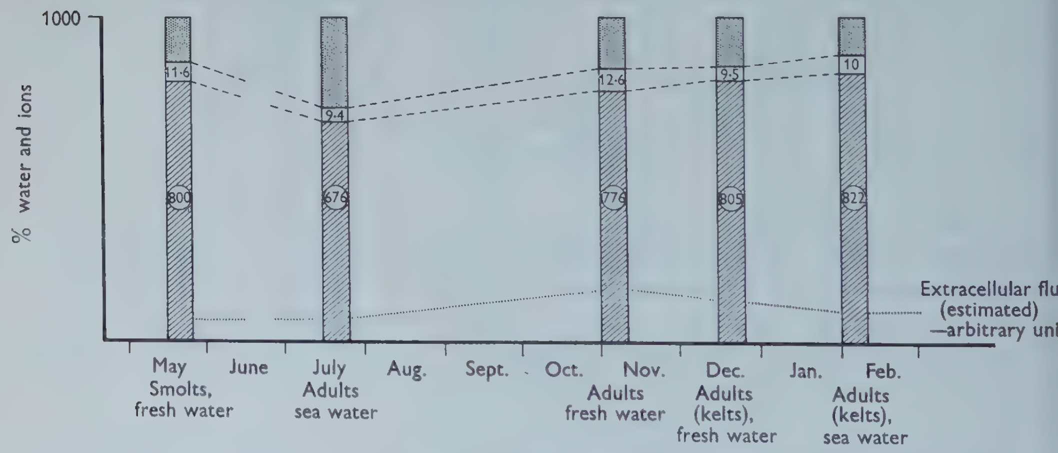

Fig. 3. A diagram of the changes in composition of the muscle of salmon at different times in its life history and in different environments. ▤, organic constituents; □, inorganic constituents (enlarged); ▨, water.

Analyses of the water content and some of the common inorganic ions which have been studied in blood plasma have been made for muscle (Table 7), and a pictorial summary is shown in Fig. 3.

If one compares, first of all, the muscle of freshwater spawning adults and the fresh-running sea-water adults, there are some quite marked differences to be seen.

The water content of the whole muscle has increased considerably, by nearly 15%, during the passage of the fish upstream in a period of about 5 months. This increased water content of the muscle, however, does not mean that the whole has become more dilute or watery, as both the sodium and the potassium content of the whole muscle has increased, the sodium by 100%, and the potassium by 25%. This increase probably means that the extracellular space of the spawning fish in fresh water has increased. At the same time the calcium content, which might be expected to be inside the muscle fibres rather than in the extracellular fluid, remains the same. That these changes come after and not before entry into fresh water is shown by the analyses of muscle from a fish at the head of the tide on its way upstream. The analyses for this fish are substantially the same as those for the sea-water fish.

Analyses of muscle from migrating smolts in fresh water show an interesting contrast, in that although the water content of the muscle is high, the sodium is low. This could be interpreted in terms of a small extracellular space, but with the usual ionic content inside the muscle, because in this case the potassium (which will be mainly inside the cells) is high. A comparison of the two measurements of chloride in the muscle substantiate this point. The figure for muscle from adult sea-living fish is about twice that of the smolt. Most of the chloride will be in the extracellular space, and thus the figures would represent a small extracellular space in the smolt, but a larger one in the marine adult.

Kelts which have been in fresh water for 9–10 months show different changes. In these fish the water content of the muscle is high (800‰) and the sodium content is mid-way between that of the young freshwater fish, and the adult spawning fish in fresh water. This could indicate an enlarged extracellular space (as in the spawning fish), but also a dilution of the muscle fibre contents, a conclusion borne out by the lower potassium content and by the drop in calcium.

Next we can consider the changes which follow the transfer of fish from one salinity to another when the fish is not in equilibrium osmotically. Two weeks after the transfer of migrating smolts to sea water the water content is scarcely reduced at all, nor does the sodium increase appreciably. Potassium, however, does drop. These changes could be interpreted as a movement of both water and ions out of the muscle fibres into a slightly increased extracellular space. It must be remembered that at the same time the blood becomes concentrated to some extent, so that the water movement could be an osmotic one and the ion extrusion from the fibres could be stimulated by the subsequent concentration within them; it is known that muscle fibres have well-developed powers of ion regulation. The slight increase in extracellular space could be the result of a time lag in these processes.

When the adult spent fish are subjected to the reverse change of salinity, the results are different. Kelts acclimatized to Morecambe Bay sea water (at a salinity equivalent to about two-thirds of oceanic water) showed a slight reduction in water content of the muscle, but retained the high sodium level. Potassium was lower than in spawning fish, but the same as in marine ones, while calcium was low, as in the spent fish. Thus, following our previous interpretation, the extracellular space is still enlarged, and this is indicated also by a high sodium content. The potassium is at the level characteristic of the fresh-run sea-living adult, so that in other respects the muscle is in equilibrium with the blood and with the external salinity, after this period of acclimatization. However, the figures for fish transferred to an artificial* sea water for much shorter periods, show a pronounced disequilibrium 3 hr. after the transfer. The water content, perhaps surprisingly, had not changed very much from the 800‰ characteristic of the kelts in fresh water, so that although there was some osmotic depletion of water from the muscle into the blood, this is not so high as one might expect from the increase in blood concentration which follows such a transfer (Δ_{blood} changes from 0·63° C. to about 1·00° C.). The potassium does not change much in this period, but the sodium content does so very sharply. Thus while the volume of extracellular space only changes to a moderate degree, its ion concentration probably does increase, possibly by the movement of sodium from the blood plasma into the extracellular space. After a longer period in the artificial sea water, the water content of the muscle increases, while the sodium declines to a more normal level and the potassium increases a little. Presumably the extracellular space increases again at this time, as osmotic control in the blood is re-established by the movement of water into the extracellular space only; some of this water may come from the muscle fibres, since their potassium content is increased. That the fish is far from achieving equilibrium conditions is shown by the marked divergences of these analyses from those for fresh-running sea fish, as well as from a study of the blood changes at this time.

Values for the extracellular space are obviously of great importance in checking the assumptions made in this interpretation. Unfortunately, while such values can be obtained for small fish in aquarium conditions, e.g. in smolts being transferred from fresh water to sea water, it is very difficult to arrange an experiment to obtain values directly for the larger adult fish.

Similar increases in extracellular space of adult *Salmo gairdnerii* recently transferred from fresh water to sea water have been reported by Houston (1959) from similar data, but Gordon (1959*a*), studying salinity transfers in *S. trutta*, thinks that the extracellular compartment is more or less constant. One of the most important differences between the experiments of these two authors and the ones described here is that the changes reported in this paper are those shown by the naturally migrating fish, at the relevant times of the year, so that the three sets of experiments may not be strictly comparable.

Sodium in the muscle of adult fish is reported to be subject to considerable seasonal and reproductive changes, e.g. in *S. trutta* (Gordon, 1959*a*) and in *Oncorhynchus nerka* (Idler & Tsuyuki, 1958). Some of the change from 66 mM./kg. in spawning fish, to 45 mM./kg. in the post-spawned fish could be due to this. It is tempting to correlate the changes during the reproductive period with changes in availability of steroid hormones; Canadian workers have found high levels of cortisone and cortisol in adult fish just prior to spawning (17 times the normal human level), while in fish before entry into fresh water the level was only one-sixth of this (Idler, Ronald & Schmidt, 1959): Spalding (unpublished, reported in Chester Jones, 1956) showed that dosing brown trout with DCA or cortisone raised the sodium and lowered the potassium in muscle. Similar changes in the concentrations of steroids in *S. salar* have been found during the parr–smolt change (Fontaine & Hatey, 1954).

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* Made by adding the appropriate quantity of Tidman's Sea Salt to Morecambe Bay sea water.

Trimethylamine oxide, while apparently absent from the blood in significant quantities, was found in the muscle to a variable extent. In post-migratory smolts still in fresh water, the concentration was 35 mg. %, in fresh-running marine adults it was 75 mg. %, and had risen as high as 250 mg. % in adult spawning fish in fresh water. Kelts in fresh water 3–4 months after spawning had very variable concentrations between 100 and 176 mg. %. The presence of any trimethylamine at all in the juvenile freshwater fish is interesting in view of the hypothesis that this substance is a characteristic of marine fish only, although it has previously been reported for freshwater fish (Anderson & Fellers, 1952). This trimethylamine is unlikely to be derived from the diet, but could represent the final stage of a basic metabolic pathway which is relatively inoperative in the juvenile fish. The measurements of trimethylamine in the adult fish are very interesting, in that the level is so high in the spawning fish. This must argue against an osmoregulatory function for this substance (Beatty, 1939) since one would expect the opposite change. It must be remembered that the spawning fish are starving, and perhaps the high level of trimethylamine present in the muscle arises from the metabolism of its own tissues during this period. The lower and extremely variable concentrations found in spent kelts could relate to their degree of debilitation.

DISCUSSION

Some specific points arising from this investigation may be mentioned. The anionic deficiency of the blood in the marine fish is of great interest osmotically, and attempts are being made to characterize this.

Nitrogen-containing compounds are possible contributors to the ion content of the plasma. Ammonia seems seldom to have been investigated in salmonids. Its concentration is likely to be low, however (reported as 0·104 mg. % in laked *Salvelinus* blood) since both the kidney and gill epithelia of teleosts are permeable to it. In teleosts generally the blood level is low. Trimethylamine or its oxide is a possible non-protein nitrogen constituent, but this was not found in any significant amounts in the plasma, even though it is present in the muscles. Betaine is another possibility which has not yet been investigated. Protein nitrogen, as amino acids, seems to be present in the blood in significant amounts. Hoar (1953) records *ca.* 70 mg./l. protein in 'mature' (?sea water) adults and a drop to 33 mg./l. in spawning fish. Recent analyses of amino acids in the blood of rainbow trout and salmon kelts indicate levels of amino acids between 30 and 60 mg./l.* How this is related to the spawning migration and to the freshwater–sea-water transfer is not yet known.

Cholesterol is another organic constituent of blood which may be important osmotically, first, in a direct concentration effect, and secondly because of its water-binding affinities. In the Pacific salmon, *Oncorhynchus nerka*, the total cholesterol in the blood is reduced from nearly 600 mg. % at the beginning of the spawning migration to 200 mg. % at the point of spawning. The pre-spawning level is singularly high in comparison with warm-blooded animals (Idler & Tsuyuki, 1958). Similar high levels have been reported for other fish (carp, 662 mg. %, Field *et al.* 1943) and for other salmonids (in a Japanese river, probably *O. keta*, 150 mg. %, Okamura 1935). The relationship between cholesterol levels and water balance would be an interesting problem.

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* Determined by C. B. Cowey, N.I.R.D., Shinfield, Reading.

The possible presence of other, unspecified constituents in the plasma should not be overlooked. Recent investigations of the osmotic behaviour of some arctic fish have shown that these teleosts can raise the total osmotic concentration of the blood to equivalence with sea water. The extra blood concentration was not contributed by the common inorganic ions, nor by glucose, glycerol, proteins, urea or ammonia (Scholander, Van Dam, Kanwisher, Hammel & Gordon, 1957).

Pre-migratory changes in water and electrolyte metabolism

Some authors, notably Kubo (1953), Pickford & Atz (1957), Fontaine & Callamand (1948) and Fontaine (1951) have reported falling plasma and tissue concentrations of chloride in pre-migratory juvenile and adult fish, and have suggested that this is a characteristic of anadromous species. Fontaine & Callamand further suggested that the electrolyte depletion plays a role in the initiation of the seaward migration by producing increased activity in the fish. Investigations by Fontaine & Baraduc (1954), Fontaine, Lachiver, Leloup & Olivereau (1948), Fontaine & Leloup (1950, 1952), Hoar (1952) and Swift (1955, 1959) of changes in the thyroid gland, and changes induced by dosing salmonid fish with thyroid hormones, indicate that the increased activity undoubtedly shown by migrating smolts is related to the activity of this gland.

On the other hand, Hoar (1953) and Nishida (1953) relate the pre-migratory decrease in chloride to the development at this stage of numbers of 'chloride-secretory cells' in the gills of the two *Oncorhynchus* species they studied, viz. *masou* and *nerka*. This hypothesis is difficult to test. In *S. salar* these cells are present in the fry as soon as the gills are functional, generally some 2 years prior to migration. Whether the number of these cells increases before the seaward migration, is difficult to establish; also one might ask if the cells are non-functional during 2 years of juvenile freshwater life. Alternatively, a suppression of the freshwater salt absorbing mechanism could occur as a pre-adaptation to marine osmoregulation, especially as certain other biochemical characteristics of the young fish change at this time to those characteristic of the adult marine fish. It seems clear from experiments described in the literature that an adjustive period is necessary after the fish has entered sea water before it can deal adequately with the different osmotic situation. Black (1957) and Houston (1959) found independently that for chum salmon fry 36 hr. is necessary for adequate regulation to be developed in sea water; Keys (1933) found a period of about 50 hr. for *Anguilla vulgaris*; Houston (1959) and I (Parry, 1958) find about 50 hr. necessary for *S. gairdnerii* in 50% sea water; and my figures for *S. trutta* of smolt size and age indicate that about 200 hr. is necessary in (50% sea water), although migrating smolts of *S. salar* were much better able to adjust to the salinity change. Following this adjustive phase, a second or regulative phase takes its place and regulation of the plasma and muscle concentrations brings the levels of individual ions to those characteristic of marine teleosts. Histological changes in the acidophil cells in the gills (Vickers, 1958) after transfer of fish to saline media, indicate a similar time for an adjustive, followed by a regulative phase. A more recent electron microscope study of these cells (Houston & Threadgold, 1961) could support this conclusion.

Thus, far from a condition of pre-adaptation to a marine life, which the falling chloraemia of the migrating smolt has been said to induce, there are indications that the young fish are unprepared, or very inadequately prepared, physiologically for this change, and require a considerable time before the regulative phase can be brought into play. Salmonids (and other euryhaline diadromous fish) differ from other teleosts in their ability to reach this regulative phase before they suffer ill-effects from the imbalance of ions in plasma and muscle in the adjustive phase, and to be able to regulate after the fashion of both marine and freshwater teleosts, according to the environment in which they find themselves.

SUMMARY

1. Osmoregulation of the Atlantic salmon in fresh water and sea water, and during transfers from one salinity to another, has been studied by measuring the freezing-point and the levels of some inorganic ions in the blood plasma, and water content and ions in whole muscle.

2. An increase in blood concentration of about 12% follows the transfer of juvenile fish (smolts) from fresh water to sea water; and a fall of concentration of about 5% follows the transfer of the adult fish from sea water to fresh water.

3. Some changes in analyses of whole muscle indicate changes in the extracellular compartment during transfers from one salinity to another.

4. Osmoregulatory powers of juvenile salmon (smolts) and fresh-run adults are good, but spent fish (kelts) returning from fresh water to sea water, osmoregulate with difficulty or not at all.

The material used for the physiological studies reported here has been obtained principally from natural sources from many different parts of Britain. Without assistance from the various River Boards and other authorities this would have been impossible, and I would like to thank all those who have been concerned. In particular, I am most grateful to Messrs G. Common, Northumberland and Tyneside River Board; H. Evans, Dee and Clwyd River Board; H. Gavin, Aberdeen Harbour Board; B. C. Lincoln, Scottish Hydro Electric Board, Invergarry; and L. Stewart, Lancashire River Board.

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TERRITORIAL BEHAVIOUR IN BARNACLE SETTLEMENT

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INTRODUCTION

When a heavy settlement of the larvae of certain sedentary marine organisms has taken place on a uniform surface, the resulting pattern may quite clearly appear to be non-random, the individual larvae having each settled at a more or less uniform distance from one another. Such a pattern is formed during the settlement of barnacles and of the tubeworm *Spirorbis borealis* (Wisely, 1960). On the other hand, some marine larvae, notably those of many Polyzoa, settle apparently at random on a uniform surface, individuals sometimes touching each other. When the settlement is less dense, the tendency for barnacles and *Spirorbis* to be spaced out may not be so obvious to the eye, presumably because the distance over which the larvae regularly avoid each other has become small in relation to the total distance separating them. Nevertheless, the avoidance of the territory around settled individuals can still be revealed by a suitable analysis of the pattern.

The simplest analysis can be made by counting the number of individuals in each of a large number of equal quadrats; if settlement is random these will fit the Poisson distribution; if the larvae show a territorial tendency the counts will be more uniform; if they are gregarious the counts will be more divergent. The significance of their deviation from the Poisson distribution may be obtained by means of the χ^2 test. A typical example of such a treatment applied to a fairly heavy settlement of cyprids of the barnacle *Elminius modestus* is shown in Table 1. There are certain difficulties in applying this treatment. It is, in practice, so difficult to provide a surface uniform

Table 1. *Distribution of* Elminius modestus *spat in quadrats* 5 *mm.*^{2} *compared with a Poisson distribution of equal population density*

No. in quadrat	Frequency observed in 187 counts	Frequency expected on Poisson distribution	χ^2
0–4	15	19·73	1·14
5	10	17·75	3·39
6	29	23·34	1·37
7	27	26·31	0·02
8	37	25·94	4·70
9	24	22·74	0·07
10	21	17·94	0·52
11	16	12·85	0·77
> 12	8	20·40	7·53
	187	187	19·51

$P = 0{\cdot}01–0{\cdot}02$

in respect of all environmental factors, that settlement is rarely uniform at the outset. Moreover, the gregarious tendency shown by some larvae, notably by barnacles, leads to an increase in settlement rate in the presence of settled individuals (Knight-Jones & Crisp, 1953) so that a high rate of settlement tends to be maintained in the neighbourhood of the first groups of individuals to become established, further settlement spreading thence outwards in course of time. Consequently, any initial unevenness in the early phase of settlement is accentuated. In making a test for territorial behaviour it is clearly necessary (*a*) to restrict counts to an area of comparatively uniform settlement, and (*b*) to use a quadrat small in comparison with the size of any patterns formed by the inhomogeneities in settlement density. With the use of quadrats of increasing size the effect of uneven or gregarious settlement is exaggerated so that the frequency dispersion increases and may eventually exceed the value expected from the Poisson distribution.

Apart from establishing a significant degree of over-dispersion, this form of analysis gives no information on the nature of the pattern which develops, and in particular fails to indicate the distance over which the repulsion between previously settled and exploring larvae operates. A far more revealing analysis can be applied by measuring the average population density as a function of the distance from any one individual. This analysis will be described both for the settlement of larvae in one dimension, along a line, and for the more usual case of settlement over a uniform area.

Analysis of linear settlement

In organisms whose larvae are attracted to grooves (rugophilic) settlement may, at least at first, be entirely constrained to a predetermined line (Crisp & Barnes, 1954). If the position of each individual along such a line were independent of that of the others and of any other factor, the arrangement would be a random linear series. If the mean linear density were ρ individuals per unit length, the chance of finding an individual in any short length dl would be $\rho\, dl$. If we now take any one individual as a reference point, we may consider how far along the line we are likely to go before reaching the next individual. The chance of finding the next individual at a point lying somewhere between l and $l+dl$ from the reference individual will be called P_0, the subscript referring to the condition that no individuals are present between the zero point and l. This probability, P_0, will be the product of two independent probabilities, namely that of not finding an individual between 0 and l, and that of finding one between l and $l+dl$. The former probability will be taken as a function of l, $f(l)$, and the latter will be $\rho\, dl$; hence $P_0 = f(l)\rho\, dl$. The probability of *not* finding an individual between l and $l+dl$ is clearly $(1-\rho\, dl)$, and therefore the probability of not finding one between 0 and $l+dl$ will be the product $f(l)(1-\rho\, dl)$. Hence

$$f(l+dl) = (1-\rho\, dl)\, f(l).$$

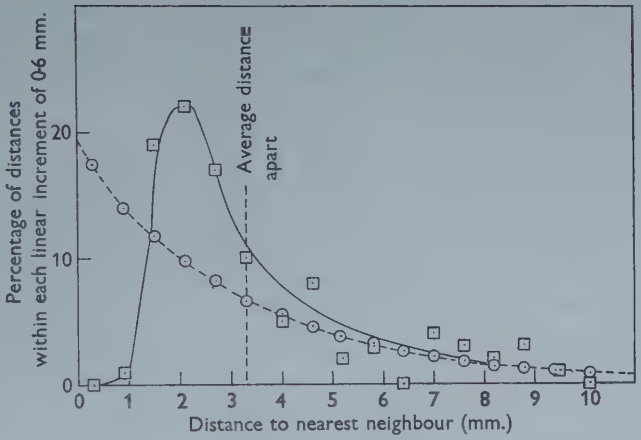
The solution of this equation with the boundary condition $f(l) = 1$, when $l = 0$ is

$$f(l) = e^{-\rho l},$$

and

$$P_0 = \rho e^{-\rho dl}.$$

This equation gives the distribution of distances separating successive individuals in a random linear series. It is illustrated in Text-fig. 1 by the dotted curve for a linear settlement of 3·05 individuals per centimetre. The full curve is drawn to connect the actual frequencies observed in a population of the barnacle *Balanus balanoides* settled at a mean density of 3·05 per centimetre along a number of grooves approximately 0·7 mm. deep and 1·5 mm. broad, with a roughly hemispherical section. It can be seen that the actual settlement differs from random in a deficiency of spacings closer than 1·5 mm. and in an excess of those in the range between 1·5 and 5·0 mm. These results correspond closely with those obtained by Wisely (1960), who investigated the linear settlement of *Spirorbis borealis* on the alga *Fucus serratus*.



Text-fig. 1. Distribution of distances between nearest neighbours in a linear series. Broken line: distribution in random settlement; full line: observed distribution for a population of *Balanus balanoides* spat in narrow grooves.

The analysis can be made more informative if we consider not simply the distribution of distances separating successive individuals, but the density of settlement as a function of distance from the reference individual.

By reasoning analogous to the above, the probability $P_1, P_2, \ldots, P_n$, of finding an individual between l and $l+dl$ from the reference point, with 1, 2 or n intervening individuals, can be shown to follow the series

$$P_1 = (l\rho)\, \rho e^{-\rho l}\, dl,$$

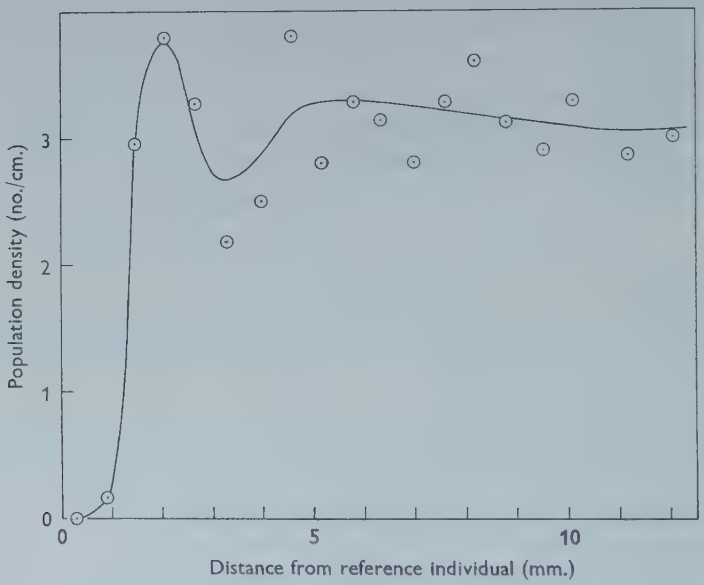
$$P_2 = \left(\frac{l^2 \rho^2}{2!}\right) \rho e^{-\rho l}\, dl,$$

$$P_n = \left(\frac{l^n \rho^n}{n!}\right) e^{-\rho l}\, dl.$$

In any short section between l and $l+dl$ the total probability of an individual being present is

$$\Sigma P_1+P_2+P_3+\ldots+P_{n=\alpha} = \rho e^{-\rho l} dl \left(\Sigma 1+\rho l+\frac{\rho^2 l^2}{2!}+\ldots+\frac{\rho^n l^n}{n!}\right) = \rho\, dl.$$

This summation is carried out in practice by taking each successive individual in turn as the reference point and counting the number of individuals in successive short intervals of $\frac{1}{2}$ or 1 mm. from it. A large number of observations are then summed and averaged over the first 10 or 20 intervals or until the influence of the reference individual can be discounted, the value of ρ becoming constant and equal to the average population value for the series. The above analysis shows that for a random settlement a constant value of ρ is to be expected. The actual observations (Text-fig. 2), taken from the same data as those from which Text-fig. 1 was constructed, show however that for this particular population density there was a low probability of settlement occurring between 0 and 0·15 mm. from the centre of any settled individual, a higher probability than average at 2 mm. and the normal value was restored between 5 and 10 mm. The dip at 3–4 mm. suggests that the regularity in spacing may extend just beyond the second individual which was most usually found at about 2 mm. from the first (reference) individual. These results are similar to those obtained by Wisely on *Spirorbis borealis*.



Text-fig. 2. Population density as a function of distance from any individual taken as a reference point for a linear settlement of *B. balanoides*.

Analysis of two-dimensional settlement

By an argument analogous to the above we may consider the settlement of individuals spaced at various distances from a central reference individual. If successive annuli of equal area, da, are drawn from the reference point and the average population density is σ, we have

$$P'_n = \frac{a^n \sigma^n}{n!}\,\sigma e^{-\sigma a}\,da.$$

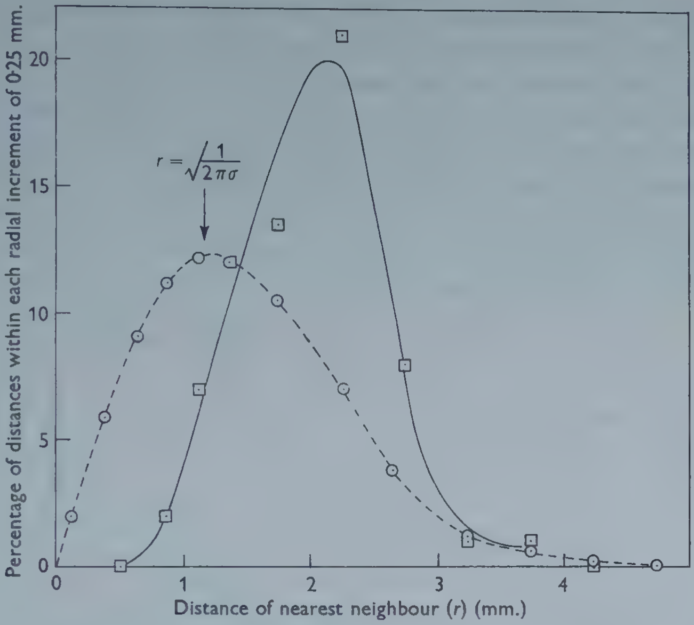
Putting $a = \pi r^2$, $$da = 2\pi r\,dr,$$

$$P'_n = 2\pi r\,\frac{\pi^n r^{2n} \sigma^\sigma}{n!}\,\sigma e^{-\pi r^2 \sigma}\,dr.$$

The distribution of distances for the individuals nearest to the central reference individual will be

$$P_0 = 2\pi r \sigma e^{-\pi r^2 \sigma} dr,$$

a result given previously by Clarke & Evans (1954) and by Thompson (1956) following Morisita (1954).



Text-fig. 3. Distribution of distances between nearest neighbours in a two-dimensional settlement. Broken line: distribution in random settlement; full line: observed distribution for a population of *B. crenatus* spat.

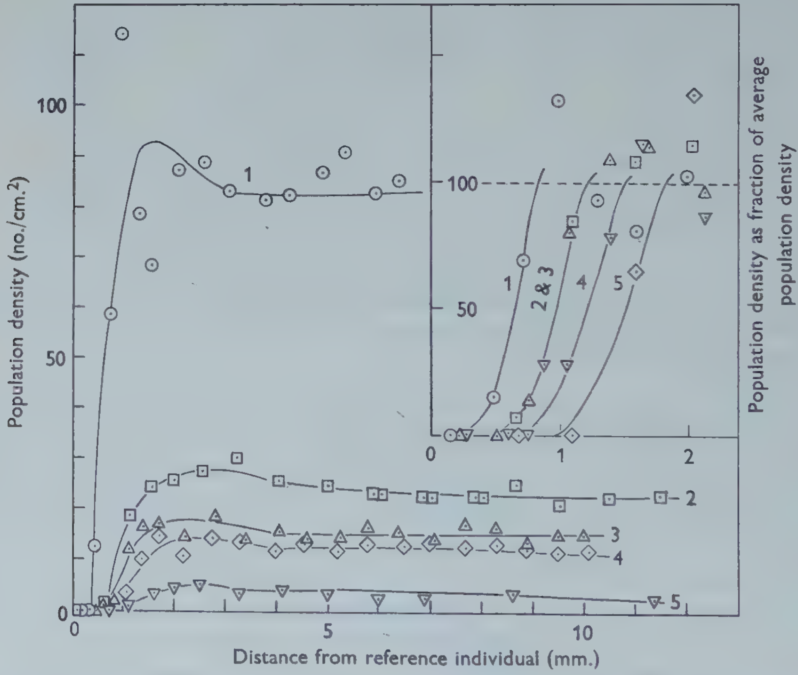
This equation therefore gives the distribution of distances between any two pairs of individuals on a plane surface. It will be seen that the relationship differs from that of the linear series in that the most probable distance between successive individuals is not zero, but finite. The modal value of P_0 will occur where $dP_0/dr = 0$, i.e. at $r = 1/\sqrt{(2\pi\sigma)}$.

Text-fig. 3 illustrates by means of a broken line the distribution of distances expected for a settlement of density 10·4/cm.^{2}. The points and the full line connecting them show the actual distribution for a settlement of *Balanus crenatus* of population density 10·4/cm.^{2}. It can be seen that there are few spacings within 1 mm. of a settled individual, while at a distance of 2–3 mm. the frequency of spacings is abnormally high, so that the modal distance is greater than $1/\sqrt{(2\pi\sigma)}$, and there is also a much more pronounced peak.

In order to illustrate more precisely the effect of an established individual on settlement, essentially the same treatment was applied as for the linear series. A large number of individuals were taken as reference points, the number present in successive annuli counted, the results pooled and averaged, and finally expressed as the number per unit area by dividing by the area of the appropriate annulus. In practice this operation was carried out on an enlarged photograph of the settlement pattern, not on the settlement itself. A transparent grid, on which successive annuli had been tinted in order to allow easy identification of the annulus, was placed over the photograph for the purpose of counting. The results of a number of such observations on different species are described below.

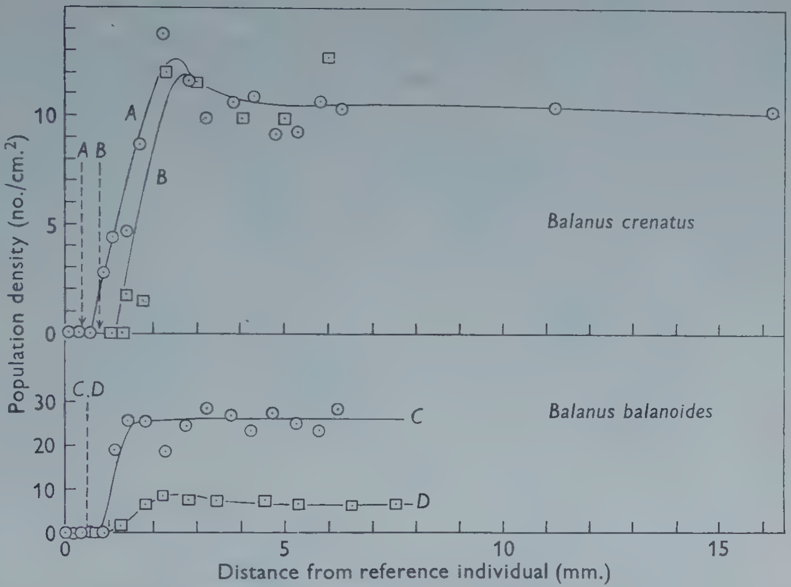
Influence of population density on territorial behaviour

As the available surface becomes more densely populated, subsequent arrivals will either have to settle closer to established individuals or else will have to search out the few unoccupied territories still available, or may do both. The influence of the density of the population on the settlement pattern is shown in Text-fig. 4 for the immigrant species *Elminius modestus*, which settles at extremely high population densities in the Essex rivers during the summer months. The curves all show a general similarity. The territory situated 1 mm. or slightly less from the reference individual is avoided, especially in the more sparse settlements. The population density then rises very sharply and at distances varying from 1 to 4 mm. reaches a value in excess of the normal level.



Text-fig. 4. Population density as a function of radial distance from any individual taken as a reference point for a series of settlements of *Elminius modestus*. Inset: the same series of curves with the vertical axis adjusted to give the population density as a percentage of the value found a long way from the reference point. Population densities were: Curve 1, 85/cm.²; curve 2, 22·5/cm.²; curve 3, 15·0/cm.²; curve 4, 12·5/cm.²; curve 5, 3·5/cm.².

Since the population density curve rises so steeply at a certain distance from the reference individual, we may reasonably use the distance between the reference individual and the point at which the steep part of the curve cuts the average value of population density as a measure of the avoidance of the territory of established spat by later colonizers. This distance, which will be referred to as the territorial separation, becomes reduced as the population density increases. This is seen more clearly in the inset graph in which the same data are presented, but the absolute population density is replaced by the population density expressed as a fraction of the normal value found at some considerable distance away from the reference individual.



Text-fig. 5. Population densities as a function of distance from an individual for settlements of *B. crenatus* (*A*, *B*) and *B. balanoides* (*C*, *D*). The dotted lines indicate the position of the boundary of already settled spat.

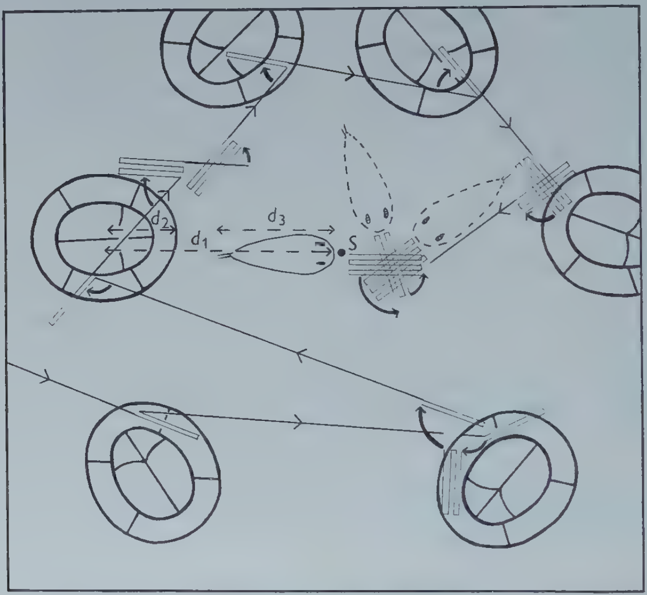
Table 2. *Territorial separation and dimensions of cyprids and spat*

Species	Density (no./cm.^{2})	Territorial separation d_1 (mm.)	Radius of spat d_2 (mm.)	Length of cyprid d_3 (mm.)	$d_1-(d_2+d_3)$ (mm.)
E. modestus	85	0·86	0·30	0·5	0·06
	22·5	1·25	0·40	0·5	0·35
	15·0	1·25	0·25	0·5	0·50
	12·5	1·42	0·35	0·5	0·57
	3·5	1·90	0·75	0·5	0·65
B. balanoides	25	1·6	0·55	0·9	0·15
	3	1·9	0·40	0·9	0·60
(linear)	3/cm.	1·55	0·45	0·9	0·20
B. crenatus	10·4	1·8	0·35	0·75	0·70
	10·4	2·3	0·85	0·75	0·70

Territorial behaviour of different species

The fact that population density influences the territorial separation between individuals complicates any comparison of the behaviour of different species. Text-fig. 5 gives results for two species, *Balanus crenatus* and *B. balanoides*, whose cypris larvae and metamorphosed spat are larger than those of *Elminius modestus*, while

Table 2 summarizes the values of territorial separation for all three species, including those of linear settlements of *Balanus balanoides* shown in Text-figs. 1 and 2. It will be seen that the species with larger larvae and spat have larger territorial separations. The two curves for *B. crenatus* were from the same settlement, using, as reference individuals, just-metamorphosed cyprids (curve *A*) and the largest spat (curve *B*). The results show that the settlement around larger spat (mean diameter 1·7 mm.) has a territorial separation which exceeds that around cyprids and just-metamorphosed individuals of diameter 0·4 mm. by a distance equal to the difference in the radius of the central individual. Evidently territorial behaviour depends on physical contact in barnacles, as it does in *Spirorbis* (Wisely, 1960), the newly settled individual having spaced itself from the edge of the previously established one. Moreover, if the mean radius of the existing spat (d_2, column 4) is added to the mean length of the cypris larva (d_3, column 5) and the total subtracted from the value of the territorial separation, the difference for all species is a little more than half a millimetre in sparse populations, and approaches zero in dense ones. It is known that during the early exploratory phase of settlement the larva explores a wide area, while towards the end of this phase, and just prior to fixation, it moves to and fro repeatedly over a small space (Knight-Jones & Crisp, 1953), moving scarcely more than its own length from its chosen position and making occasional swivelling movements (Text-fig. 6). These final movements would cause it to keep at a distance of at least its own length from the edge of any outstanding object, such as its own spat, unless it deliberately settled in the angle between the object and the surface. This pre-settlement behaviour can therefore adequately account for the values of territorial separation given in Table 2.

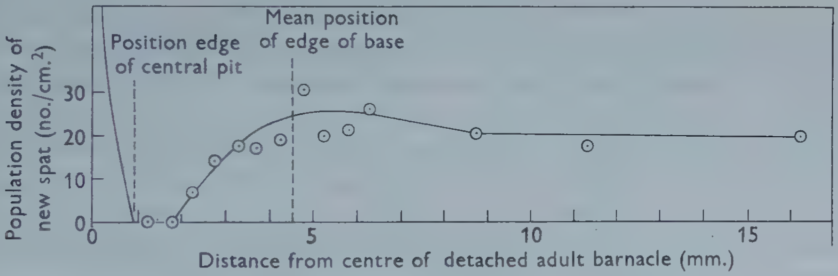


Text-fig. 6. Movements of cyprids of *Balanus balanoides* just before fixation (semi-diagrammatic). Straight lines with arrows represent normal translation, to and fro movements are shown conventionally by thinner parallel lines, and swivelling movements by thick curved arrows. d_1, territorial separation; d_2, radius of existing spat; d_3, length of cypris larva.

If there were sufficient space available few individuals would chance to settle in the position shown in Text-fig. 6 with the caudal setae as close as possible to another individual. If the amount of space had become very restricted, as in a dense settlement, such a site might be the only suitable one to be found, and so an increasing proportion of individuals, even after a long search, would lie within this distance of each other.

Influence of the base of detached specimens

When a settling cyprid encounters a small barnacle it may receive two kinds of stimuli: a simple physical stimulus, which would be invoked by touching any similar object, and a more specific stimulus, possibly chemical, through which it might recognize the object as another barnacle. When a barnacle is removed from a surface it usually leaves behind its base, which remains firmly cemented in position. Knight-Jones (1953) demonstrated that barnacle cyprids could recognize these bases, as well as the living barnacle, and if they belonged to the same or related species settlement was encouraged (Knight-Jones, 1955). Hence we may assume that if the base were flat it could be specifically recognized, though the physical stimulus it exerted would be small.



Text-fig. 7. Variation in population density of spat of *B. balanoides* settled over the adherent base of individuals which had been removed, as shown in Pl. 1.

A number of plates of inert plastic, on which small pits had been drilled in order to localize settlement, were placed in the sea and one barnacle was allowed to settle and grow in each pit (see Crisp, 1960*a*). After the barnacles had grown to about 1 cm. in diameter the pits were usually to be found near the centres of the bases. The basal membrane covered the pit, but did not fill it up, and the membrane itself replicated the concavity. The barnacles were then cleaned off the panels, leaving behind the bases, together with a few strands of muscle tissue. These were then exposed in the sea to settlement during the following season. Pl. 1 shows the appearance, both of the original settlement of adult barnacles and of the later spat in identical positions on two of the panels. It can be seen that the new settlement was very dense in the membrane-covered pits situated roughly at the centre of each base, and another dense zone of settlement took place at the periphery of the base. The remainder of the base was generally sparsely colonized.

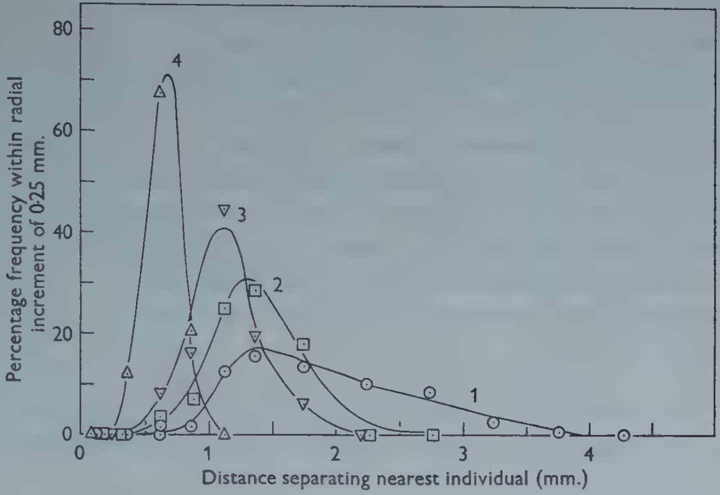
Text-fig. 7 shows population density as a function of distance from a reference point situated in the position of the pit where the adult first settled, after averaging a series of counts over some twenty-five bases. The dense settlement in the original pit is shown by the peak on the vertical axis; then follows a region of low settlement density; finally the curve rises to a further peak at a distance of 4 mm., approximating to the radius of the base of the adult shell. The low value of population density just outside the pit may be caused in part by the territorial influence of the spat settled in the pit, but this cannot account for settlement densities less than the average at distances of up to 4 mm. from the pit, because the territorial influence does not normally extend beyond 2 mm. (Table 2). Moreover, a few adult barnacles did not settle over a pit, and their bases therefore did not include a concavity (Pl. 1, *X*, *Y*). These also showed sparse subsequent settlement within the basis, though they lacked the dense central colony. These experiments indicate that the centre of the base tends to be avoided, but the periphery to be heavily colonized. While this behaviour suggests that there may be a specific response to the integument causing the barnacle to avoid the base of a detached individual or one of its own species, there remains the possibility that the centre of the base or the outside of a small individual may have a physical contour that is unfavourable for settlement in comparison with other surfaces nearby.

Territorial behaviour in pits

Concavities in the surface are preferentially colonized by cyprids (Crisp & Barnes, 1954). When more than one cyprid enters a small pit, it is forced to settle very close to the original occupant. Hence within such a pit one would expect territorial behaviour to be reduced or eliminated. In some instances cyprids can be seen to have settled so close together that some are unavoidably in contact with small spat of their own species. However, it is possible that the behaviour leading to the recognition of and settlement in a pit may be separate from the recognition and avoidance of a previously settled individual. If this were so, the two elements of behaviour might play opposing roles during the colonizing of a small pit.

An experiment was devised to investigate whether there was any tendency to 'space out' when settlement occurred within a small pit. Into a sheet of inert plastic were drilled out three groups of hemispherical pits having different radii of curvature. Twelve of each of the different sized pits were randomized into groups of 36, and each of these groups were replicated three times. After drilling, the pits were polished with fine carborundum followed by jewellers' rouge, then scrubbed with a detergent solution, washed in water, and exposed to a settlement of *Balanus balanoides*.

The areas within the pits were so limited in extent and curved in shape that it was impossible to make counts at various distances from each individual. Instead the distances between neighbouring individuals were measured, and their frequencies plotted to find whether the modal value differed from that calculated from random expectation. Text-fig. 8 shows the series of curves obtained for a plane surface and for each of the series of pits of increasing curvature. The shift of the curves towards smaller values of separating distances as the curvature of the pits was increased indicates the concentration of spat in the smaller pits under identical conditions of settlement. The modal value of each frequency curve is shown in Table 3, together with the value expected for a random arrangement within the pit. It will be seen that, even at the highest population density, the cyprids appear to be spaced out more than would be expected from chance. However, at the higher population densities, the expected modal distance is so close to, and in one case less than, the sum of the radii of two metamorphosed spat (0·6–0·7 mm.) that the apparent spacing-out effect might be due only to the fact that two adjacent small barnacles cannot overlap each other. An alternative method was therefore employed to confirm that territorial behaviour was displayed during settlement in pits.



Text-fig. 8. Distribution of nearest neighbours in a settlement of *B. balanoides* on surfaces of various curvatures. Curve 1, on plane surface; curve 2, in pit of radius of curvature 13·5 mm.; curve 3, in pit of radius of curvature 6·5 mm.; curve 4, in pit of radius of curvature 1·5 mm.

Table 3. *Distances separating individuals in pits of various curvature after exposure to the same spatfall of* Balanus balanoides

Radius of curvature of pit (cm.)	Curve in Text-fig. 8	Mean settlement density (no./cm.^{2})	Modal value of distance from nearest neighbour in Text-fig. 8 (mm.)	Expected modal distance for random settlement $\frac{1}{\sqrt{(2\pi\sigma)}}$ (mm.)
Plane	1	10	1·5	1·26
1·35	2	33	1·25	0·69
0·65	3	45	1·15	0·59
0·15	4	85	0·65	0·44

A number of pits of different sizes were drilled out on a plastic sheet to form a pattern of squares containing 36 pits, each pit being 1 cm. from the next. The pits were so arranged that each occurred once in each row and once in each column, forming a 6 × 6 Latin square design. Three such square patterns of pits were repeatedly exposed to settlement by *B. balanoides*, and the number of cyprids and spat counted. The experiment was continued until an average of 2 to 3 spat were present in the smallest pits and 6 to 7 in the largest; the pits were then cleaned out and the experiment repeated. In this way it was possible to obtain for each type of pit a series of curves showing how the proportions of pits containing zero, one, two, three, etc., individuals varied as the average number per pit increased. If settlement were random, the curves would follow the Poisson series of distributions, and the variance between counts would equal the mean number per pit. If the territorial effect were infinitely strong, all the pits would be filled by one individual each before any were entered by a second, and so on. The value of the variance would then always lie between 0 (when the average number per pit was an integer) and 0·25 (when the average number contained half an integer). A simple presentation of the results is shown in Table 4 where the proportions of pits with 0, 1, 2, 3, etc., individuals are given for a mean settlement of 1·0 and 2·0 individuals per pit respectively. At these relatively low values of settlement density there was adequate space in each pit, and particularly in the larger pits, for further individuals to settle. This is clear from the columns giving the dimensions of the various pits and the maximum number observed in each pit at high settlement density. The figures show that the distribution is very different from the Poisson; the central frequencies are much higher. Even in the largest pits there was only a slight approach towards the values predicted for chance settlement. The variance increased in the larger pits, but was everywhere well below the Poisson value. Evidently the existence of an individual settled in a pit greatly reduced the chance of a second entering the same pit.

Table 4. *Distribution of numbers of* Balanus balanoides *cyprids among pits of various sizes at an average settlement rate of* 1·0 *and* 2·0 *individuals per pit*

(The predicted values for random settlement and for an infinite repulsion between individuals within a single pit (complet territorial behaviour) are given in the last two rows.)

				Mean settlement = 1·0 per pit.					Mean settlement = 2·0 per pit.					
				Percentage of					Percentage of					
Type	Area (mm.²)	Radius, i.e. distance centre to rim (mm.)	Maximum no. observed in pit of this type during experiment	Zeros	Singletons	Doublets	Others	Variance	Zeros	Singletons	Doublets	Triplets	Others	V
A	5·03	1·0	3	12	77	11	0	0·23	0	22	56	22	0	
B	10·15	1·5	6	23	55	21	1	0·48	0	24	52	24	0	
C	12·80	1·9	6	19	62	17	2	0·40	0	15	68	15	2	
D	25·3	2·4	8	24	56	18	2	0·49	1	20	56	18	5	
E	50·7	3·7	10	20	62	16	2	0·44	0	24	51	22	3	
F	89·2	4·9	9	27	51	17	5	0·64	6	14	55	22	3	
Random settlement (Poisson)				37	37	22	4	1·00	13·5	27	27	18	14·5	
Completely territorial behaviour				0	100	0	0	0	0	0	100	0	0	

OBSERVATIONS ON SETTLING CYPRIDS

Cyprids of *B. balanoides* were obtained from plankton hauls during April and observed in the laboratory under a low-power binocular microscope as they explored and settled on various surfaces presented to them. The cyprids were presented with the following surfaces: barnacled stones, both smooth and rough; barnacled stones with various densities of young spat already settled on them; flat plastic sheets covered in small hemispherical pits spaced 0·5 mm. apart of radius of curvature of approximately 1 mm.; and flat plastic sheets with grooves approximately 1 mm. wide and ½ mm. deep containing a few spat. In one experiment on the pitted surface, each pit contained an average of two spat, in the other experiment the pits had been cleared, leaving only the bases of the spat behind. Each cyprid was carefully watched and timed to find out how long it spent in exploring, what proportion of this time was spent within pits and grooves, and whether the exploration terminated in settlement. The cyprids showed two types of movement on the antennules, a simple translatory step which took them on a more or less straight course through a distance of about 0·5–

Table 5. *Behaviour of cyprids on various surfaces*

Condition of surface	Barnacle density (spat + adults) (no./cm.^{2})	No. of explorations observed	Mean time per exploration (min.)	Maximum time of an exploration (min.)	Percentage of explorations ending in a settlement (%)
Bare surfaces					
Smooth plastic	0	12	0·8	3·1	0
Rough stones	0	5	0·9	1·6	0
Barnacled surfaces					
Rough stones with spat	0·2–3·0	6	13·8	24·0	67
Rough stones with spat and adults	2·0–5·0	5	8·6	35·3	20
Rough stones with spat and adults	> 10	5	9·7	29·7	0
Grooved and pitted surfaces					
Grooves with very few spat	0·1	16	6·9	42·8	6
Pits with bases of spat only	0	6	25·2	55·0	80
Pits with dense spat	4·0*	9	36·6	92·2	11

* Expressed per unit area of curved surface of pit the value would be 30–40.

0·75 mm., and a less deliberate movement, characterized by pivoting and oscillating on the antennules, often accompanied by a change of direction, which will be called a 'halt'. Translatory steps generally predominated during the early phase of settlement, and halts towards the end. Table 5 records the length of time of the exploratory phase and shows very clearly the influence of previously settled spat or their bases. These provide the necessary stimulus which causes the cyprids to remain on the surface and to continue to search for suitable sites on which to settle. However, although this response was common to all surfaces which were barnacled, the best settlement occurred where a low density of settled spat (0–3/cm.^{2}) was present on the surface. Where the density was more than 10/cm.^{2} a clear reduction in the tendency to settle was evident.

The time that was spent exploring the surface was not greatly affected by the presence of dense spat. In fact, the exploration time on panels containing pits was greater when spat had already occupied the pits than when the pits were vacant. The cyprids could be seen entering the occupied pits one after another, exploring them and walking on to the next, apparently seeking an empty one. Indeed the only occasion when a cyprid was observed to settle on this type of panel took place when it encountered a pit which happened to be unoccupied. The number of times that cyprids entered pits, walked out and entered others is shown in Table 6.

The exploration time of the cyprids which were presented with the grooved panel containing only a very few spat in one corner was much less. This could be attributed to the small number of spat, which resulted in few of the larvae encountering them, the majority making short and fruitless searches which terminated in their swimming away from the surface. Those which did encounter spat remained for about the same length of time as those on more heavily barnacled surfaces, as can be judged from column 5 which gives the maximum times.

Table 6. *Behaviour of cyprids when presented with surface depressions containing previously settled spat and their bases*

Surface	Spat density (no./cm.^{2})	Total no. of times pits and grooves visited	No. of cyprids settled	Mean no. of pits or grooves visited before settlement	Mean no. of pits or grooves visited per excursion
Plastic containing grooves with few spat	0·1	158	1	158	9·9
Plastic containing pits with bases only	0	80	5	16	13·3
Plastic containing pits with dense spat	4·0	271	1	271	30·1

MECHANISM OF TERRITORIAL BEHAVIOUR

Unlike the territorial behaviour of active animals, in which the holder of a territory usually displays aggression, that of sedentary animals must be attributed solely to the behaviour of the invading individual, through which the territory of the first is respected. It can hardly be doubted that the observed separation is brought about by the characteristic short to and fro excursions and the twisting movements that take place repeatedly just before fixation.

The territory avoided by later arrivals has been shown to be quite limited in range. It does not usually extend beyond a circle of radius 2 mm. from the previously settled individual. Of this distance 0·3–0·5 mm., according to the species, must be allowed for the radius of the central individual and a similar distance for the radius of the new arrival because the shells of the two individuals obviously cannot interpenetrate. Only the observation of a regular separation of individuals beyond 0·6–1·0 mm. is therefore of consequence. Such separations, though small, are nevertheless real and may be retained even at population densities as high as 20 to 30 individuals per square centimetre.

In spite of its very short range, territorial behaviour is particularly interesting and significant because it seems contradictory to the thigmotropic tendency displayed by cyprids when they settle preferentially in grooves and pits. One might expect from their reaction to concavities that they would settle in close contact with other individuals, nestling in the angle between them and the substratum. However, it is not certain that cyprids settle in depressions simply in response to increased physical contact. There are a number of reasons for believing that they may be capable of recognition of surface contour. First, they do not settle into a pit as soon as they come to it, but explore it, entering it or similar pits many times. Secondly, pits and grooves with very large radii of curvature attract cyprids although the increased contact compared with a plane surface must be very small indeed. Thirdly, cyprids avoid sharp angles and prominences, just as they seek out depressions.

The junction between a small barnacle and the substratum offers an internal angle in the vertical plane, but it is strongly convex in the horizontal plane. The angle does not therefore provide a greatly increased degree of physical contact since the cyprid can only make contact tangentially with the small barnacle. The cyprid might instead respond as it would to a convex rather than to a concave surface, avoiding it and settling a little way off. The base of a large barnacle, on the other hand, is much less convex; indeed a species such as *Elminius modestus* with a sinuous octaradiate outline has eight concavities which are depressed in both planes, and so are highly attractive, often being occupied by a small barnacle in each.

If the reaction of cyprids to contact with other individuals were clearly different from their reaction to an inert surface, the mechanism of territorial behaviour would involve, in addition to the physical response to an obstacle, the specific recognition of other barnacles as their own species and a change of behaviour in consequence. Recognition of their own species has been clearly shown by Knight-Jones (1953) to exist in barnacles. Knight-Jones & Moyse (1960) also find that, while the cyprids of certain barnacles avoid contact with individuals of their own species, they settle directly in contact with barnacles of other species. This clearly implies that specific recognition of the surface of their own species is the basis not only of gregarious but also of territorial behaviour. Some of the observations reported in this paper lend support to the view that the three species studied avoid to some extent settling on the surface of their own species. The sparse colonization of the membranous base suggests a specific response. It is difficult to explain why cyprids do not settle readily into a pit containing young spat without assuming that they recognize them as barnacles. Such a pit, even though it contains a small barnacle, remains essentially a concavity. At the bottom of the pit, where the small barnacle generally lies, a groove is formed between it and the pit wall. When a pit is drilled with a blunt twist drill a similar configuration is produced at the bottom of the pit, but in this case it is of inert material and the cyprids settle readily all round the groove. The similar groove formed by the sides of a barnacle already settled at the bottom of the pit is avoided, however, and later cyprids entering usually settle high up on the walls of the pit. However, pits retaining the basis of a small barnacle, but otherwise empty, seem very attractive. Perhaps, therefore, both a convex surface contour and the presence of a barnacle surface produce the strongest avoiding reaction.

Not all barnacles avoid settling on adults of their own species. Cyprids of *Balanus hameri* settle predominantly on the sides of the adults, forming in time groups of barnacles growing out in a tree-like form from an older basal individual (Moore, 1935). In the season of settlement of this species the adults are covered with young spat to a greater degree than the *Modiolus* shells on which they commonly grow. *Chelonobia patula*, an epizoic barnacle found in America predominantly on *Callinectes sapidus* also settles heavily on its own adults, not only on the sides but also just in the angles of the opercular aperture. They seem commoner on their own species than on the cuticle of the crab itself. Young individuals of *Lepas anatifera* and *Pollicipes cornucopiae* settle very close to or even on the stalk of other individuals, though they avoid the valves. In another pedunculate genus, *Scalpellum*, the complemental male must settle on the valves of hermaphrodite specimens. All these examples are species which are obviously gregarious; it follows that territorial behaviour during settlement is entirely independent of gregariousness. These species also resemble each other in having an isolated or restricted habitat, and in not spreading out over it to the same extent as rock-living balanoids.

The three species investigated in this paper behaved very similarly at settlement, and seemed to possess the same basic mechanism. If soon after alighting the cyprid came in contact with its own species it would continue to explore the surface for a long time. If it did not encounter its own species it was likely to swim off. Towards the end of its exploration, when it was about to settle, contact with its own species caused a renewal of exploring and testing behaviour until the cyprid obtained a more acceptable position nearby, such as an unoccupied depression.

It is necessary to postulate only that contact with its own species stimulates further walking to account both for gregariousness over a wide area, and territorial behaviour at short range. Early in the pre-settlement phase contact with another barnacle would cause the cyprid to walk and explore widely, so that it eventually settled somewhere in the vicinity. Towards the end of settlement, when the activity of the cyprid was mainly confined to halting and pivoting, contact stimulation by its own species would similarly cause it to explore further; but, because of its slower progress, it would not move far before attempting to settle again (Fig. 6).

THE SIGNIFICANCE OF TERRITORIAL BEHAVIOUR

The only obvious adaptive advantage in spacing out during settlement is to allow the individuals sufficient space for growth. It seems clear that the behaviour of the cyprid during the immediate presettlement phase, when it meticulously explores by random steps, and tests with the antennules an area within 3–4 mm. of its final settling position, is an adaptation to ensure that the immediate vicinity is free of all objects that might interfere with settling or with its later growth. From the point of view of the growth of the individual, the cyprids could with advantage space themselves much farther apart, since they are capable of growing to a diameter of over 1 cm. during the first season. However, if the cyprid were too exacting in its requirements in comparison with cyprids of other species, it would be likely to leave enough space for the latter to grow in between itself and its neighbours. There are three serious disadvantages to any species of barnacle that allows a second species to interpenetrate. The second species would reduce the available surface for the first species to colonize. It would expose the first species to the danger of being crushed or overgrown (Connell, 1959). Finally, if interpenetration were considerable and the first species were cross-fertilizing, it could result in a proportion of individuals being isolated and consequently sterile. It is thus clear that the cyprids cannot afford the risk of spacing out sufficiently far apart to give themselves enough space to grow to the maximum size. The optimal distance apart is that which is as near as possible to each other consistent with retaining a reasonable chance of survival to maturity. After a heavy spatfall, therefore, many small barnacles will be too close to survive. However, there are two mechanisms which accommodate to some extent the varying numbers of individuals that survive the spatfall. During growth, an individual which is close-packed can modify its shape from that of a flat cone to that of a tall narrow column, without apparent harm. Perhaps more important, once established, an individual or group of individuals under pressure can slide slowly over the substratum to fill up odd spaces, or to make room for other growing individuals (Crisp, 1960*b*). Hence it seems likely that the most critical phase for survival occurs during or just after metamorphosis, while the shell plates are not fully formed, hardened, and cemented to the substratum. It is at this time that the impact of another growing individual might be disastrous, since the very small specimen might be crushed rather than pushed along. The interval of time from fixation of the cyprid to the completion of the shell plates and the beginning of growth in girth is in *B. balanoides* about 1 to 1½ weeks. The rate of growth of fast-growing young barnacles is about 1 mm. per week (Barnes & Powell, 1953; Crisp, 1960*a*). Hence a spacing out of about 1 mm. from established individuals would allow just enough time for subsequent spat to establish themselves, but would probably make very insecure any individual that attempted to settle in between. Territorial behaviour is therefore to be expected in those dominant forms which spread out flat during growth, covering the substratum to the exclusion of other species. Forms which exist as small isolated groups in unusual habitats are relatively free from competition; they need not therefore become adapted to monopolize the available surface and so may not need to protect themselves against being squeezed off by spacing out during settlement.

SUMMARY

1. An analysis of the natural pattern of settlement of barnacles, whether arranged along a groove or over a plane area, shows that later settlers tend to maintain a distance of about 2 mm. from earlier ones. This phenomenon in a sedentary form is analogous to territorial behaviour in active species.

2. A method is described which gives average values for population density as a function of the distance from any reference individual. It is found that the reference individual lies in a trough of very low population density, and that the latter rises sharply a short distance away. The distance from the reference individual, at which the population density reaches the average value for the whole population, is defined as the territorial separation.

3. The territorial separation is reduced, but not entirely eliminated, as the population density rises.

4. The value of territorial separation is a little greater than the sum of the radius of the previously settled spat and the length of the cyprid, and is slightly smaller in species with smaller cyprids and spat.

5. Cyprids settle at the periphery of the base of detached specimens rather than at the centre.

6. Though strongly attracted to pits in the surface, cyprids continue to behave territorially within a pit, and avoid pits containing previously settled individuals. They settle readily in pits with only the bases of detached individuals present.

7. Observations on settling cyprids show that after encountering settled barnacles they tend to remain and explore the surface. If they continue to encounter settled individuals at the time they are about to settle, the period of final searching is prolonged until they find a suitably vacant area.

8. Territorial behaviour is probably an adaptation allowing the recently metamorphosed cyprid time to establish itself before it touches the growing edge of another barnacle.

I am indebted to Prof. E. W. Knight-Jones and Mr J. Moyse for allowing me to read their manuscript on intraspecific competition in sedentary marine invertebrates while this paper was still in the press.

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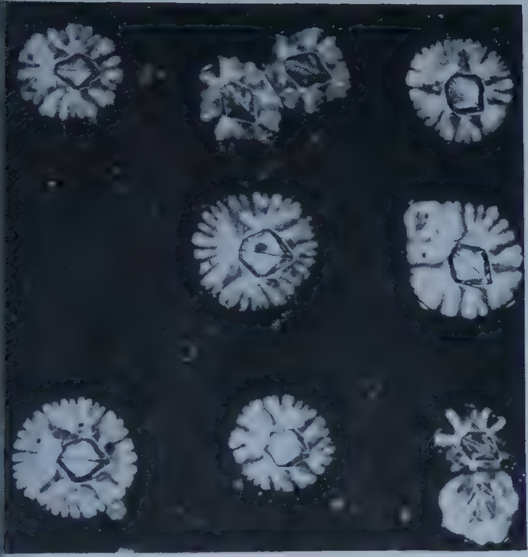
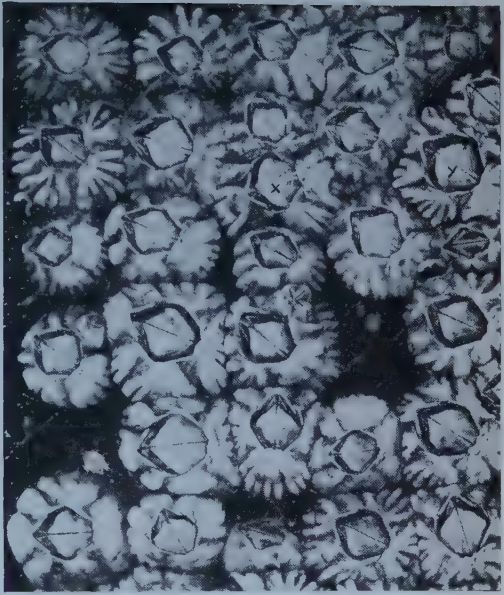
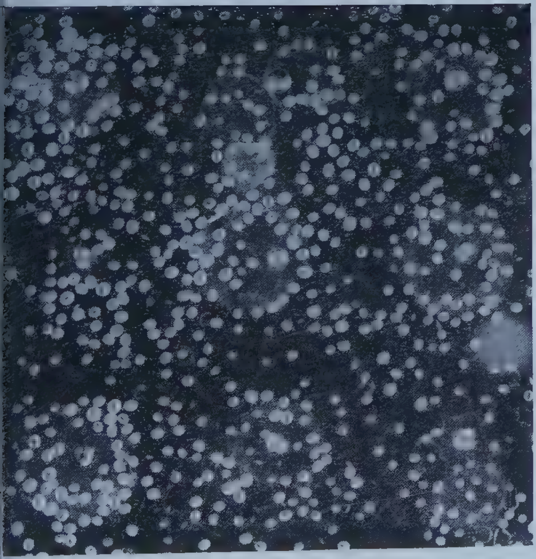
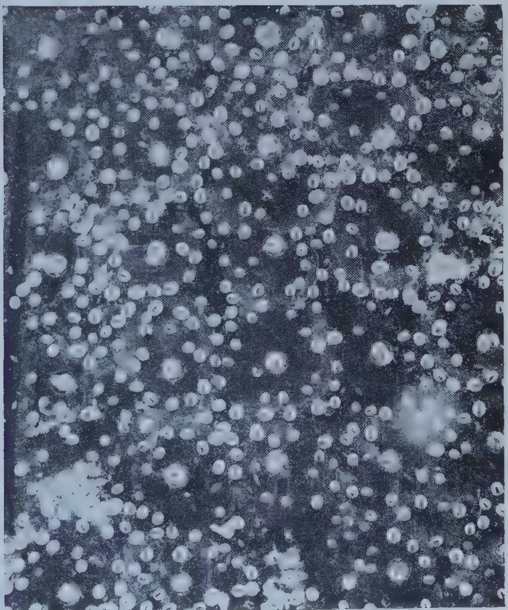
EXPLANATION OF PLATE

The upper pair of photographs show a settlement mainly of *Balanus balanoides*. These were removed, leaving their bases behind. These can be seen in identical positions in the lower pair of photographs. The newly settled spat tend to avoid the bases (except for the depression in the middle of each base) and settle at the periphery of the base or outside it. *X* and *Y* are bases without central pits. The left-hand photos include specimens of *Elminius modestus* in pairs at the top centre and bottom right.









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EFFECT OF DISSOLVED OXYGEN CONCENTRATIONS ON THE TOXICITY OF SEVERAL POISONS TO RAINBOW TROUT (*SALMO GAIRDNERII* RICHARDSON)

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(*Received* 20 *January* 1961)

INTRODUCTION

The low dissolved oxygen concentrations which are characteristic of many polluted rivers have been shown by several authors to increase the toxicity of poisons to fish. Since most toxicity tests are made in well aerated water, it is important to know what factor to apply to the results when predicting the effect of a reduced dissolved oxygen concentration on toxicity. Evidence presented in this paper suggests that there may be a common relation between the dissolved oxygen concentration and the toxicity of poisons, and this is supported by a theoretical consideration of the problem.

METHODS

Toxicity of monohydric phenols, and zinc, lead and copper salts

Details have been published of determinations of the effect of various levels of dissolved oxygen concentration on the toxicity of a mixture of monohydric phenols (Department of Scientific and Industrial Research, 1958), zinc sulphate (Lloyd, 1960), lead nitrate and copper sulphate (Department of Scientific and Industrial Research, 1960).

Toxicity of ammonium chloride

The effect of low dissolved oxygen concentrations on the toxicity of ammonium chloride to rainbow trout was determined in fixed volumes of solution; pH values and oxygen concentrations were controlled by aeration with known mixtures of air, nitrogen, and carbon dioxide. The trout were fed before they were acclimatized for 18 hr. to the temperature (17·5° C.) and carbon dioxide concentration used in the tests. Other details of procedure were similar to those described by Lloyd & Herbert (1960).

Oxygen consumption of rainbow trout

Measurements of the oxygen consumption of rainbow trout at 17·5° C. were made in a simple continuous flow respirometer, similar in essentials to that illustrated by Fry (1957) in his figure 10B. Values for oxygen consumption were first obtained in water saturated with air and the same fish were immediately used again to obtain oxygen-consumption values at a lower oxygen level. The rainbow trout used weighed between 1 and 11 g. and were acclimatized to the temperature and free carbon dioxide concentration (about 8·0 mg./l.) before the test.

RESULTS

Monohydric phenols, and zinc, lead and copper salts

When the log. survival times of rainbow trout are plotted against the corresponding log. concentrations of these poisons in well aerated water, a curvilinear relation is obtained, and at those concentrations of the poisons in which periods of survival are long the line is nearly vertical, so that a further slight decrease in concentration is associated with a prolonged period of survival. It is these slightly toxic concentrations of poisons which are important for predicting safe concentrations in a river. If the dissolved oxygen concentration of the water is reduced, the survival time/concentration curve is displaced towards lower concentrations of poison, and a value for this increase in toxicity can be obtained by comparing concentrations of poison which are equitoxic at prolonged periods of survival. This can be expressed as the factor X_S/X, where X_S is the concentration of poison at 100% of the air-saturation value of oxygen, C_S, and X is the equitoxic concentration at a lower value of dissolved oxygen, C. Values of X_S/X at different levels of dissolved oxygen concentration were derived from the experimental data for monohydric phenols, and zinc, lead, and copper salts for median periods of survival between 1000 and 2000 min. Values of X_S/X for these four poisons are shown in Fig. 1, where it appears that the relation between increase in toxicity and dissolved oxygen concentration is similar for these poisons.

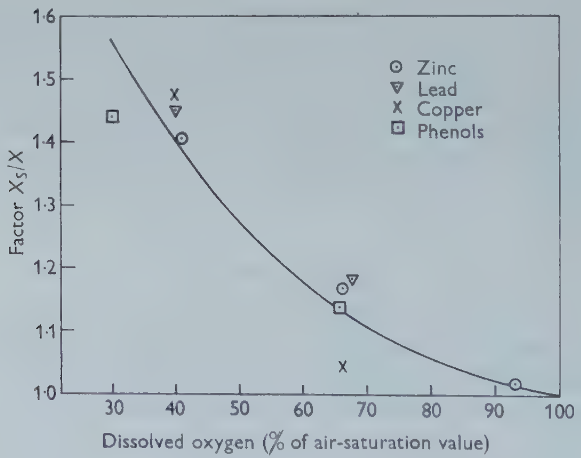

Fig. 1. Relation between the factor X_S/X for several poisons and the dissolved oxygen concentration of the water. For explanation of X_S/X see text.

Ammonium salts

Batches of ten rainbow trout were exposed to various concentrations of ammonium chloride at two levels of free carbon dioxide (3·4 and 19·8 mg./l.) and three levels of dissolved oxygen (37·5, 66·0 and 100% of the air-saturation value); concentrations of ammonia corresponding to 500-minute median periods of survival (at which time the survival time/concentration curve has become practically vertical) were calculated by probit analysis for each series. The experimental values of X_S/X for ammonia

(Fig. 2) are higher than those for the previous four poisons, and are affected by the free carbon dioxide concentration of the water; these differences can be explained by the following hypothesis.

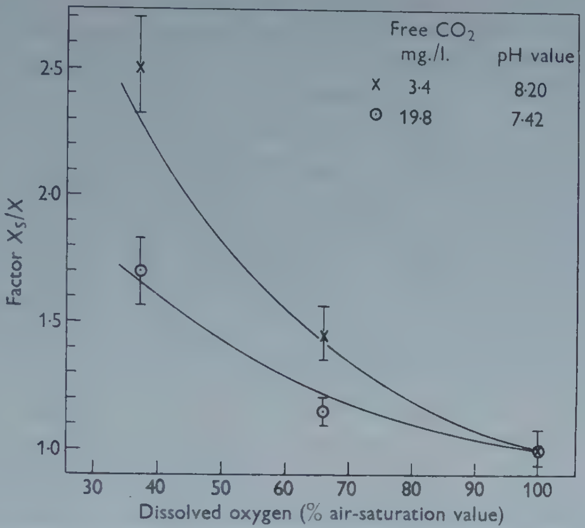

Fig. 2. Relation between the factor X_S/X for ammonia and the dissolved oxygen concentration of the water at two levels of free carbon dioxide. Continuous lines are theoretical curves (see text). Limits to experimental points are 95 % fiducial limits.

It is well established that the toxicity of ammonia solutions is due to the un-ionized ammonia molecule, and that the ionized fraction is not toxic; the un-ionized proportion of an ammonia solution increases with a rise in pH value. However, it has been shown by Lloyd & Herbert (1960) that the toxicity of ammonium salts is dependent, not on the pH value of the bulk of the solution, but on that of the water at the gill surface. This latter value can be calculated from the bicarbonate alkalinity, temperature, and free carbon dioxide concentration in the water, and the free carbon dioxide excreted by the gills of the fish. An estimate of the concentration of excreted carbon dioxide in the respiratory water (as mg. carbon dioxide/l.) is given by the following relation

$$\text{D.O.} \times \text{R.Q.} \times \frac{\text{mol wt. } CO_2}{\text{mol wt. } O_2} \times \frac{P}{100}, \qquad (1)$$

where D.O. is the dissolved oxygen concentration of the water in mg./l., R.Q. the respiratory quotient of the fish (assumed to be 0·8), and P the percentage of oxygen removed from the respiratory water by the fish; the values of P used are given later in the discussion. As the oxygen concentration of the water is reduced, the concentration of excreted carbon dioxide at the gill surface is also reduced and the pH value of the water at this surface rises, resulting in an apparent increase in the toxicity of ammonia. This increase in toxicity will become greater as the concentration of free carbon dioxide in the bulk of the solution is reduced.

Thus, theoretical values of X_S/X for ammonia can be calculated on the assumption that the relation between dissolved oxygen concentration and the toxicity of this poison is essentially similar to the relation for the other four poisons (Fig. 1), but that in water of lowered oxygen content the toxicity is further increased, because of the reduction in the concentration of excreted carbon dioxide at the gill surface. An estimate of this additional increase in toxicity can be derived from the theory given by Lloyd & Herbert (1960). Theoretical curves for the factor X_S/X for ammonia under the conditions of the experiments described here are shown in Fig. 2 where they are in good agreement with the experimental points. From data in a paper by Merkens & Downing (1957) on the effect of a reduction of the dissolved oxygen concentration to 47% of the air-saturation value on the toxicity of ammonia, it can be calculated that the experimental factor for X_S/X for a 500 min. median period of survival was 3·64; the factor expected for the experimental conditions, in which the concentration of free carbon dioxide was between 0·75 and 1·0 mg./l., is between 3·35 and 4·17. The good agreement between predicted and experimental results strengthens the view that the effect of low dissolved oxygen concentrations on the toxicity of this poison is basically similar to that for the other four poisons, but that its toxicity is increased still further by the rise in pH value of the water at the gill surface.

DISCUSSION

Although the toxic actions of heavy metals, ammonia, and monohydric phenols are probably dissimilar, the common effect on their toxicity resulting from a reduction in the concentration of dissolved oxygen suggests that this is a result of a physiological reaction by the fish to such a change of the environment, and is independent of the nature of the poison. The most obvious reaction of fish to a lowered oxygen content of the water is to increase the volume of water passed over the gills, and this may increase the amount of poison reaching the surface of the gill epithelium, the site at which most poisons are absorbed. Weiss & Botts (1957) have shown that an increase in the oxygen uptake of several species of fish results in a decrease of their survival times in toxic solutions; they found, however, that a reduction in the dissolved oxygen concentration of the water reduced the oxygen uptake of the fish, yet increased the toxicity of the solution, and thought that this reduction in uptake was insufficient to compensate for the reduced oxygen content of the solution and that it was the increased rate of respiratory flow through the gills which led to an increased toxicity of the poison. However, the design of their experiments does not allow the results to be compared in detail with those from the experiments described here. Therefore, although there is some evidence that an increase in respiratory flow increases the toxicity of poisons, there is no evidence to show that this accounts for the whole of the increase in the toxicity of poisons in water of low dissolved oxygen concentration. The following hypothesis is suggested to explain the relation between respiratory flow and the toxicity of poisons.

The structure of the teleost gill has been described in detail by other authors and has been summarized by Fry (1957); essentially it consists of a sieve of fine plates which form long narrow channels (about 20 μ wide in rainbow trout) through which the respiratory water flows. It is assumed that in such a fine capillary system, and over the normal range of respiratory flow rates, the flow pattern will be laminar, even though the respiratory current may not be continuous but intermittent (Hughes & Shelton, 1958). Since the walls of this channel (the respiratory epithelium) form an absorbing surface for poisons, there will be a diffusion layer at this surface in which a concentration gradient of toxic substances could exist. Although there are no data on the relation between velocity of flow and the rate at which ions or molecules reach an absorbing surface in capillary systems, Štráfelda (1960) has shown that in wider tubes (a few centimetres in diameter), under conditions of laminar flow and with a constant concentration of solute, the relation conforms to an equation which may be written

$$x' = A + B\sqrt{v'}, \tag{2}$$

where x' is the concentration of solute at the surface, v' is the velocity of flow, A is the concentration of solute at the surface when v' is zero, and B is a constant for the system. It is assumed that this equation can be applied to capillary systems of the same dimensions as those existing in a teleost gill; it is also assumed that under conditions of zero flow, the diffusion layer would be of infinite depth and the solute would have to diffuse through the capillary system from the bulk of the solution outside, so that values of A would be very small when compared with the values of x' obtained with a very thin diffusion layer at normal flow rates. Therefore, the term A will be neglected, and the equation rewritten as $x' = B\sqrt{v'}$. Thus, if x_1 is the concentration of solute at the surface when the velocity of flow is v_1, and x_2 the concentration of solute at the surface when the velocity is increased to v_2, the factor for the increase in concentration of the solute at the surface, x_2/x_1, is equal to $\sqrt{(v_2/v_1)}$. Also, since it can be assumed that, within the range of concentration of poisons used in these experiments, the ratio between x' and the concentration of solute in the bulk of the solution is a constant for any given value of v', it can be shown that if the flow is increased from v_1 to v_2 and the concentration of x' is to remain at the level x_1, the concentration of solute in the bulk of the solution would have to be multiplied by the factor x_1/x_2. Therefore, if the effect of low dissolved oxygen concentrations on the toxicity of poisons is to increase the rate of respiratory flow from v_S at the air-saturation level of dissolved oxygen to v at a lower level, the decrease in concentration in the bulk of the solution required to maintain a constant concentration of poison at the surface of the gill epithelium—X/X_S—should equal $1/\sqrt{(v/v_S)}$, or $X_S/X = \sqrt{(v/v_S)}$.

It would be difficult to measure directly the velocity of water flowing through the respiratory channels of the gills, but since the dimensions of these channels presumably remain constant with small differences in the flow rates, the velocity of flow will vary directly with the volume of respiratory water passed through the gills. Volumes of respiratory water passed in unit time can be calculated from the oxygen uptake of the fish, the oxygen content of the water and the percentage removal of oxygen from the water by the fish, the equation being

$$V_S = \frac{Q_S}{C_S}\frac{100}{P_S}, \tag{3}$$

where V_S is the volume of respiratory water (l./hr.), Q_S is the oxygen uptake of the fish (mg./hr.), and P_S is the percentage removal of oxygen from the respired water when the dissolved oxygen concentration at the air-saturation value is C_S (mg./l.). Similarly, at a lower level of dissolved oxygen, C, $V = 100Q/CP$ where V, Q and P are the velocity of flow, oxygen uptake of the fish and percentage removal of oxygen from the respired water respectively at the lower level of dissolved oxygen. Therefore, the increase in the rate of respiratory flow when the dissolved oxygen concentration of the water is reduced from C_S to C is given by the equation

$$\frac{V}{V_S} = \frac{C_S P_S Q}{C P Q_S}. \qquad (4)$$

Values of Q_S and Q were obtained from respirometer experiments with rainbow trout at two dissolved oxygen levels, C_S and C, and are given in Table 1; values for P_S and P have been given by Van Dam (1938) for the same species (see Fig. 4). These values were used to calculate the factors for $\sqrt{(V/V_S)}$ shown in Fig. 3, where they are compared with the curve fitted to experimental data for X_S/X, shown in Fig. 1. The close relation between the points for $\sqrt{(V/V_S)}$ and the curve for X_S/X lends support to the hypothesis that the increased toxicity of poisons at low dissolved oxygen concentrations is the result of an increased concentration of poison at the surface of the gill epithelium, and that the concentration of poison in the bulk of the solution has to be reduced from X_S to X to maintain a constant concentration of poison at that surface.

Table 1. *Oxygen uptake of rainbow trout at different levels of dissolved oxygen at 17·5° C.*

Wt. fish (g.)	100 % air saturation. Oxygen uptake (mg. O_2/hr.)	Lower oxygen level: % air saturation	Lower oxygen level: Oxygen uptake (mg. O_2/hr.)
0·99	0·50	48·0	0·33
1·05	0·58	63·0	0·47
2·10	1·10	42·0	0·68
3·04	1·75	40·8	1·19
11·10	3·18	47·5	2·22

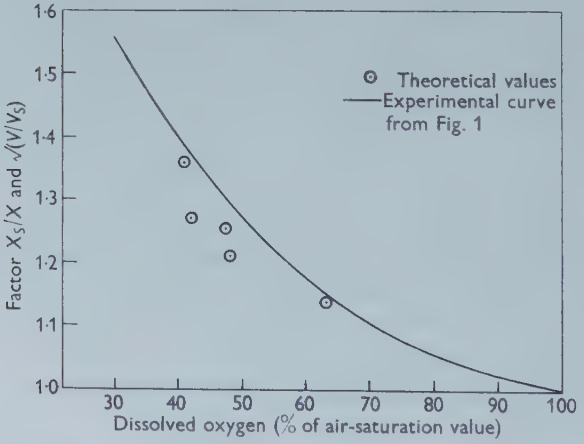

Fig. 3. Relation between the curve fitted to the experimental factors for X_S/X and theoretically determined factors for $\sqrt{(V/V_S)}$ based on the increased rate of flow of respiratory water.

However, although the agreement between these theoretical points and the experimental curve in Fig. 3 is reasonable, the values of $\sqrt{(V/V_S)}$ are all slightly lower than would be expected from the experimental curve; this discrepancy may be the result of using the values of P given by Van Dam who obtained them from a rainbow trout which was held in a clamp and which was respiring at a rate close to the standard metabolic rate, whereas the rainbow trout used in the present experiments were free-swimming and presumably respiring at a greater rate. Theoretical values of P required to bring the values for $\sqrt{(V/V_S)}$ on to the curve for the factor X_S/X are shown in Fig. 4, where they are compared with Van Dam's data. Since it is reasonable to suppose that the values of P depend upon the rate of respiratory flow, it follows that the curve relating P to C for free-swimming fish should be displaced towards higher values of C when compared with the curve obtained for clamped fish. However, at the asphyxial level of dissolved oxygen for rainbow trout (about 20% of the air-saturation value) the oxygen uptake of both free-swimming and clamped rainbow trout will be the same (Shepard, 1955), and the curve drawn through the theoretical points in Fig. 4 has been fitted on the assumption that both curves should coincide at that level. If it is accepted that the theoretical values of P shown in Fig. 4 are more likely to apply to free-swimming fish than the curve given by Van Dam, then the slight discrepancy between the calculated factors for $\sqrt{(V/V_S)}$ and the curve for the factor X_S/X in Fig. 3 is explained. The theoretical values of P were used in equation (1) to calculate the theoretical increase in the toxicity of un-ionized ammonia with a reduced dissolved oxygen concentration of the water; the reasonable fit of these curves to the experimental points in Fig. 2 lends some support to the assumption that these values of P are valid for free-swimming rainbow trout.

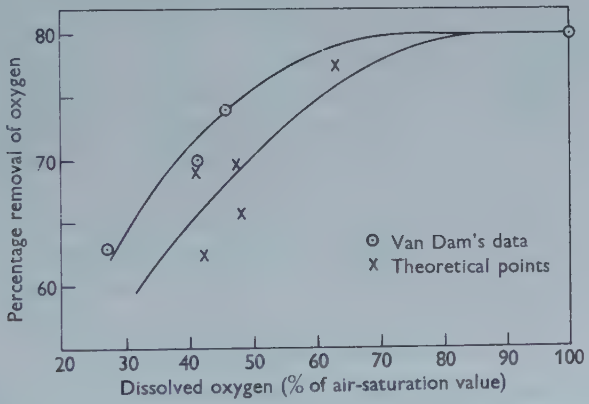

Fig. 4. Relation between the percentage removal of oxygen from the respiratory water by rainbow trout and the dissolved oxygen concentration of the water.

An estimate of the increase in the concentration of poison at the gill surface resulting from a reduction in the dissolved oxygen concentration of the water can be obtained by another approach. It may be assumed that the relation which governs the rate at which toxic molecules diffuse from the bulk of the solution to the surface of the gill epithelium also governs the diffusion of oxygen molecules. Thus, as the rate of respiratory flow is increased, so the rate at which oxygen molecules reach the epithelial surface will also increase if the dissolved oxygen concentration in the bulk of the solution remains constant. If it is assumed that the oxygen uptake of the fish is proportional to the dissolved oxygen concentration at the surface of the gill epithelium, then if the oxygen uptake of the fish was reduced from Q_S to Q, the rate at which oxygen molecules, and also toxic substances, reached that surface would also be decreased by the factor Q/Q_S. However, if the decrease in oxygen uptake was accompanied by a decrease in the dissolved oxygen concentration of the water from C_S to C, the rate at which other molecules or ions reached the epithelial surface would not be altered by the factor Q/Q_S but by the factor C_SQ/CQ_S. Values for the factor C_SQ/CQ_S, calculated from data given in Table 1, are shown in Fig. 5 where they are compared with the curve fitted to the experimental points for X_S/X. Although these theoretical points follow a curve similar to that given by the experimental factors for X_S/X, the values are somewhat higher, and it may well be that the assumption that the oxygen uptake of the fish is proportional to the oxygen concentration at the gill epithelium is not accurate. It has been suggested that at low dissolved oxygen concentrations in the water the haemoglobin content of the blood is increased (Shepard, 1955) and the rate at which blood is pumped through the gills may also be raised, both of which would increase the rate of removal of oxygen from the gill epithelium. This may account for the higher values obtained for the increase in the rate at which toxic substances reach the gill epithelium when calculated by this method.

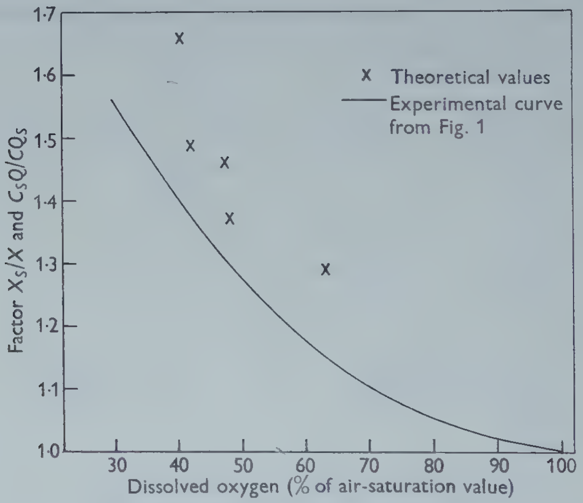

Fig. 5. Relation between the curve fitted to the experimental factors of X_S/X and theoretically determined factors for C_SQ/CQ_S based on the oxygen consumption of rainbow trout.

Neither of the theoretical methods used here to calculate the relation between the increase in toxicity of poisons and the reduction in the dissolved oxygen concentration entirely agree with the experimental data, and although the discrepancies can be reasonably explained in qualitative terms, there are no data available whereby the extent of these differences can be quantitatively accounted for. Furthermore, there would be some difficulty in obtaining the required data on the percentage removal of oxygen from the respiratory water, and on the rate at which oxygen is removed by the blood from the gill epithelium, since the measurements would have to be obtained from free-swimming rainbow trout. Nevertheless, the close approximation of the points given by the two theoretical methods to the practical values obtained for X_S/X suggests that the majority, if not all, of the increase in toxicity of poisons in water of low dissolved oxygen concentration is caused by the increase in the rate of respiratory flow.

This is of fundamental importance in fish toxicology, since it implies that any environmental or physiological change which affects the rate of respiratory flow of a fish will also affect the concentration of poison at the surface of the gill epithelium, and that a known relation exists between these two factors. It also implies that the relation between the increase in toxicity of poisons to fish and a reduced dissolved oxygen concentration of the water will be the same for all poisons except those whose toxicities are affected by the pH value of the water. Thus, the curve obtained for the factor X_S/X in Fig. 1 should apply to the effect of dissolved oxygen concentration on the toxicity of most poisons to rainbow trout.

SUMMARY

1. A given reduction in the dissolved oxygen concentration of the water from the air-saturation value to a lower level increases the toxicity to rainbow trout of zinc, lead and copper salts, and of a mixture of monohydric phenols, to about the same extent.

2. The effect of a reduced oxygen concentration on the toxicity of ammonia solutions is greater than that found for the other four poisons; the extra increase can be accounted for by a theoretical calculation of the difference between the pH value of the bulk of the solution and that at the gill surface.

3. An hypothesis is presented to account for the effect of low oxygen concentrations on the toxicity of poisons to fish. It assumes that a given toxic effect is produced by a specified concentration of poison at the gill surface, and suggests that this concentration is governed not only by the concentration of poison in the bulk of the solution but also by the velocity of respiratory flow.

The author wishes to acknowledge the assistance given by H. T. Mann and A. C. Wakeford with the experimental work. This paper is published by permission of the Department of Scientific and Industrial Research.

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THE STRUCTURE AND FUNCTION OF THE PROTHORACIC SPINE OF THE DESERT LOCUST, *SCHISTOCERCA GREGARIA* FORSKÅL

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(*Received* 25 *January* 1961)

INTRODUCTION

The family Catantopinae (= Cyrtacanthacrinae) of the acridid grasshoppers have a raised tubercle or spine on the sternum of the prothorax, between the prothoracic legs. Its shape is typically a short, thick, slightly backwardly directed spine, with many hair sensilla; this is the condition in *Schistocerca* (Pl. 1, A, B). In other genera, however, it is relatively larger (e.g. *Tropidacris*) or nearly absent (*Mesopsera*), blunt (*Tropidopola*) or very sharp (*Opshomala*): in some it is of entirely different shape, e.g. bifurcate in *Goniae australasis*, or mushroom-shaped in *Noliba elegantula*.

Uvarov (1928) commented that, while no investigations had been made, the structure had no obvious biological significance. Chauvin (1939) described the gross anatomy of the spine in *Schistocerca*, and did some extirpation experiments, but reached no conclusions as to its function.

MATERIAL AND METHODS

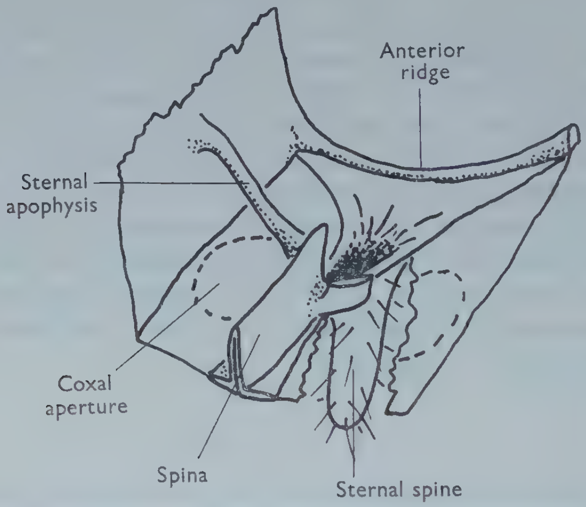
Schistocerca gregaria from laboratory culture has been used for most of the work herein described, but the much larger *Ornithacris turbida* has also been used, particularly for anatomical work. The nucleus of the latter culture was kindly supplied by the Anti-locust Research Centre.

Detailed dissection of the nervous system, especially of adult *Schistocerca*, is hindered by a tough sheath of connective tissue, tracheae and fat body around nerves and ganglia. For this reason dissection of fine sensory nerves was only practicable in material which had been fixed for some months in Bouin's alcoholic fluid to which had been added a little glycerin. This dissolved the fatty material, leaving only a thin fibrous sheath, and did not make the tissues too brittle.

The prothoracic spine and neighbouring structures are heavily cuticularized. Later instars were therefore killed soon after moulting, and embedded in ester wax, which produces minimal hardening. Sections were silver-stained by the methods of Holmes (Carleton & Drury, 1957), Samuel (1953), or Betchaku (1960), after fixation in Carnoy's, Davenport's (Samuel, 1953) or Bouin's aqueous or alcoholic fluids, or one of various formol mixtures. Some material was stained in bulk by a modification of Holmes's technique. All these combinations gave successful results on occasion, and none was completely reliable. The most consistent method was fixation in Davenport's solution for several days, followed by Samuel's method with 4 hr. in the impregnating solution.

For fibre counts, fixation was in 2% osmium tetroxide saturated with picric acid, 3 days at 0° C., and subsequent staining in iron haematoxylin.

Electrical activity in the ventral nerve cord was recorded with hooked silver/silver chloride electrodes and in smaller nerves with hooked stainless steel electrodes, 20 μ in diameter, set in Araldite about 100 μ apart. Saline was removed from the nerve/electrode junction with a fine pipette, and replaced when recording was satisfactory by a drop of liquid paraffin. As long as this did not enter the tracheal system, the preparation lived for up to 2 hr. Sensilla were stimulated either by a fine glass needle attached to the armature of a relay, or by puffs of air controlled in duration and intensity by relay-operated taps. The movements of the former were calibrated with a phototransistor, and the puffs of air measured by directing the jet at a diaphragm microphone, both feeding into the oscilloscope amplifier.

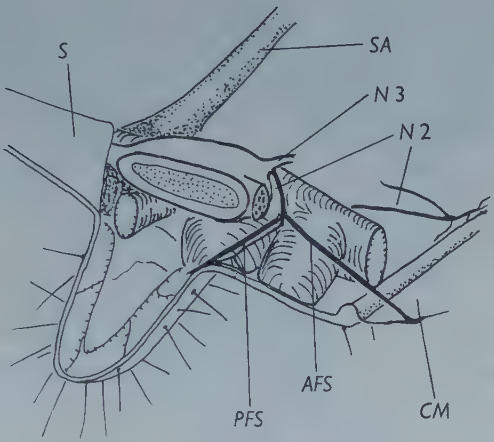


Text-fig. 1. Cuticular structures of the sternum of the prothorax. On the right-hand side the sternal apophysis and part of the coxal membrane is cut away to show the sternal spine.

STRUCTURE AND DEVELOPMENT

The sternum of the prothoracic segment carries a roughly Y-shaped area of dense cuticle (Text-fig. 1). The tail of the Y is the *spina*; near its anterior end the *sternal apophyses* run sideways and upwards as flying buttresses to the pleural walls, and from their point of attachment to the sternum two ridges run to the left and right anterior corners of the segment, where they fuse with the transverse *anterior ridge*, which forms the anterior margin of the segment and joins the tips of the Y. At the junction of the arms, at the tip of the spina, is a depression in the sternal floor which leads backwards and downwards to form the sternal spine. The outside of the spine bears (in *Schistocerca*) about 100–140 symmetrically distributed, long, articulated setae, up to 0·8 mm. long, and a few much smaller setae, about 0·05 mm. long. No other sensilla are present. Similar hair sensilla are present on the rest of the sternum and proximal leg joints (Pl. 1, A, B).

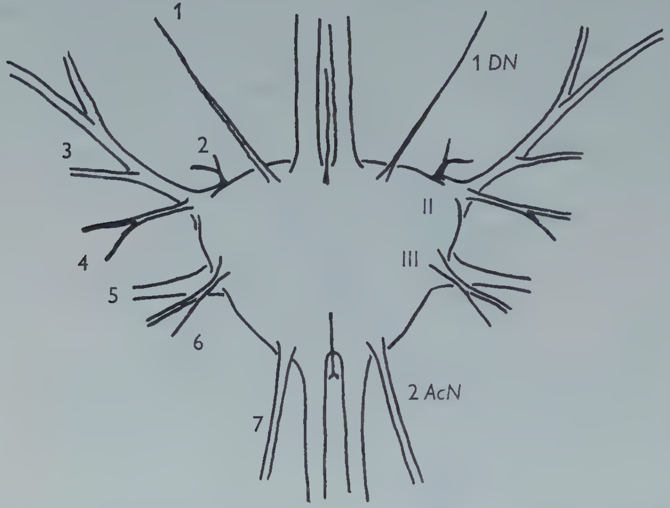
Under the sternal cuticle lies a single layer of ectodermal cells, and on this a layer of small, flattened, irregular air-sacs, bound together by connective tissue—this layer extends to the tip of the spine. On top of these small air-sacs lie the *longitudinal ventral tracheae* (*LVT*). Posteriorly they run with the posterior nerve commissures between spina and apophyses, and on the inner surface at the anterior end they feed two pairs of large air-sacs, which end blindly on the sternal floor anterior to the spine. The more posterior (second) pair lie under the segmental ganglion, which is supported on a cushion of minor air-sacs and tracheae fed from the dorsal surfaces of the *LVT* (Text-fig. 2).



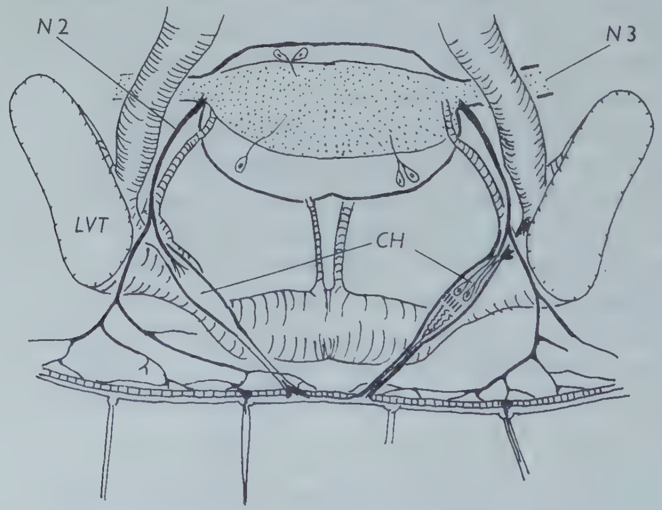
Text-fig. 2. Sagittal section of sternum to show course of nerve 2, and associated structures. *AFS*, anterior fibrous strand; *CM*, cervical membrane; *N2*, *N3*, nerves 2 and 3; *PFS*, posterior fibrous strand; *S*, spina; *SA*, sternal apophysis. The anterior commissure is shown cut away at the ganglion.

The nerves leaving the ganglion are shown in Text-fig. 3. The sternal sensilla are supplied solely by nerve 2. The nerve emerges from the ganglion together with a small trachea, and both run vertically downward to the inner surface of the *LVT*, at the junction of air-sacs 1 and 2 (Text-figs. 2, 3). Here the nerve enters a patch of connective tissue attached to the tracheal wall. From this patch an *anterior fibrous strand* runs forward to an insertion on a specially thickened patch in the midline of the cervical membrane. The nerve divides inside the connective tissue. The larger branch turns under the *LVT*, and emerges between the main tracheal trunks supplying the legs. It runs to the anterior ridge, where it splits up, supplying the sensilla along the anterior and lateral margins of the sternum. A subsidiary branch runs backwards to the leg, and supplies hair sensilla on the coxa and femur. The smaller branch runs downwards and slightly backwards from the *LVT*, together with a *posterior fibrous strand*, which is inserted distally on the cuticle at the anterior base of the sternal spine. From this point the nerve branches freely over the ectoderm to the sensilla of the spine and mid sternum.

The spine is absent in the first instar, and develops progressively at each moult. In the first two instars the tracheal system is rudimentary, but the nerve has the appropriate relations with the tissue blocks inside which the tracheae develop. By the third instar (Text-fig. 4) the main tracheae are complete, but the second nerve is still not divided into two main branches as in the adult. From the point of contact with the *LVT* the nerve branches many times and supplies all areas of the sternum. The nerve also supplies a large chordotonal organ, containing about fifteen sense cells. This is attached by connective tissue to the point of contact of nerve and *LVT*, and is inserted on the cuticle at the anterior base of the sternal spine. In the first instar the chordotonal organ is fully formed and innervated, but as the *LVT* are not yet



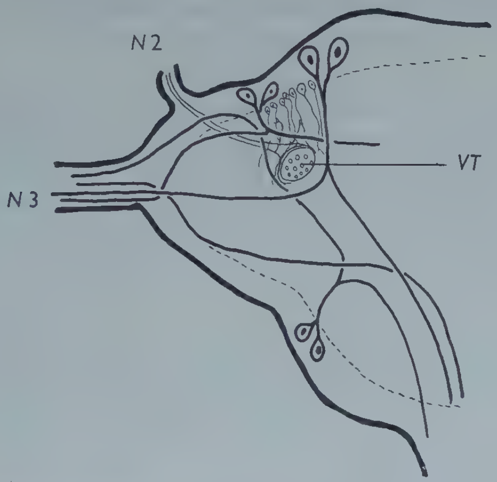
Text-fig. 3. Nerves leaving the prothoracic ganglion of *Schistocerca*, viewed from above. The arabic numerals correspond to Pringle's (1939) nomenclature of the metathoracic ganglion of *Periplaneta*, which is adopted in the text; the roman numerals and letters correspond to Schmitt's (1959) nomenclature for the prothoracic ganglion of *Dissosteira*. Nerve 2 descends vertically from the ganglion, and is not in fact visible from above as shown. 2 *AcN*, anterior ganglionic connective; 1 *DN*, dorsal nerve; II, III, second and third roots.



Text-fig. 4. Transverse section of the prothoracic sternum of third instar *Schistocerca*, showing course of nerve 2. It supplies paired chordotonal organs, and sends ramifying branches to all sternal sensilla. From serial sections, 20μ, Holmes silver stain. *CH*, chordotonal organs; *LVT*, longitudinal ventral tracheae; *N*2, *N*3, second and third nerves.

formed, its origin is diffuse. In the fifth instar the chordotonal sense cells often appear to be degenerating, and in the adult all that remains is the posterior fibrous strand, which preserves the origin, relations and insertion of the original organ. Exceptionally the organ does not degenerate, and remains apparently functional in the adult. The anterior fibrous strand is by contrast fully developed and non-nervous throughout life.

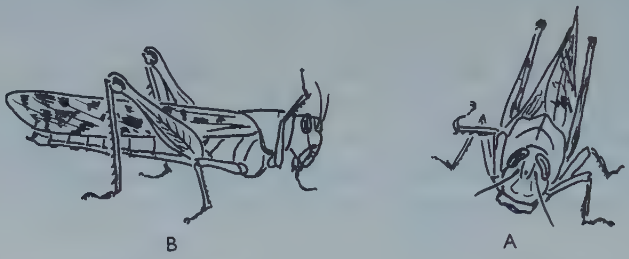
As the insertion of the chordotonal organ is on rigid cuticle, it can only monitor movements of the point of origin, i.e. the *LVT*, relative to the insertion. Such movement could possibly occur during moulting (perhaps a reason for degeneration after the final moult), but it is more likely that the normal function is to register movement of the cervical membrane, transmitted to the *LVT* by the anterior fibrous strand.



Text-fig. 5. Ganglionic connexions of nerve 2 (diagrammatic). Heavy lines, motor axons; medium lines, afferent fibres from nerve 2; fine lines, internuncial neurones. *N*2, *N*3, second and third nerves; *VT*, vertical tract of fibres running eventually to the suboesophageal ganglion. Further explanation in text.

The second nerve is shown in transverse section in Pl. 1, C, immediately before it enters the ganglion. It contains some thirty-seven fibres of 5–9 μ diameter, and seventeen smaller ones down to 1 μ. The paired second nerves, with their total of 108 fibres, supply in all about 600–700 sensilla, so it is clear that in many cases the axons of several sense cells must fuse to form a single fibre. This is confirmed by silver-stained sections. The lateral branch of the nerve supplies only its own side of the segment, but in the midline each nerve receives fibres from both sides of the spine and sternum. It has not been possible to decide whether axons from both large and small sensilla ever fuse.

The path of the fibres from nerve 2 within the prothoracic ganglion is shown diagrammatically in Text-fig. 5. They appear to synapse soon after entering the neuropile with internuncial neurones which are associated with the motor fibres of, at least, nerve 3, and also make connexion with a vertical tract of fibres which continue into the anterior connective and thus to the suboesophageal ganglion. No connexion with fibres running to the posterior connectives was found. These established connexions, though certainly incomplete, would fit in well with the behavioural and physiological results described below.



Text-fig. 6. Postural disturbances following waxing of the sternal spine, 3 hr. after operation. Further explanation in text.

BEHAVIOURAL EXPERIMENTS ON FUNCTION

Extirpation of the sternal spine by cautery under anaesthesia in the second or later instars produced no obvious effect on the subsequent behaviour of the animals; this agrees with the results of Chauvin (1939). If, however, the spine of an adult locust was covered with low melting-point paraffin (40° C.) without anaesthesia, similar disturbances were seen in a significant number of cases. The movements of the prothoracic legs appeared to be disturbed; in extreme cases the legs would 'windmill' when walking, making very exaggerated stepping movements (Text-fig. 6A). When stationary, these animals would often stand with one or both prothoracic legs off the ground. (Text-fig. 6B). (These effects are also seen more commonly in animals which have one or both commissures sectioned between the prothorax and head; the disturbances are then permanent.)

These effects soon vanished, usually within a few hours, sometimes within 30 min. If the waxing was performed under CO_2, or less drastically, N_2 anaesthesia, the effect was masked by the general lack of co-ordination and disability during recovery, and was gone by the time recovery was complete. Direct recording from nerve 2 confirmed that the wax coating stopped the sensilla functioning in response to normally effective stimuli.

The results suggest that the animal is deriving proprioceptive information relating to the prothoracic legs from the spine sensilla, but that other sources are available, as the animal quickly adapts to its loss. The long sensilla on the spine touch hairs on the coxae and mesothorax, and can presumably signal movement of these parts relative to the prosternum (Pl. 1, A, B).

SEGMENTAL REFLEXES

Stimulation of the sensilla of the sternum in the intact animal does not produce very useful results because the response of the animal varies greatly with circumstances, especially the degree to which the animal is disturbed by experimental conditions. If much disturbed, as when restrained and strongly illuminated, it would ignore the most violent stimulation, e.g. cautery, and continue its attempts to escape. In more favourable conditions light stimulation with a fine glass needle produced grooming movements of the prothoracic legs, or movement away from the needle.

When the ventral nerve cord is sectioned, both anterior and posterior to the prothoracic ganglion, a precise and strictly repeatable system of reflex responses is seen. Movement with a needle of one or more sensilla on the spine produces an effective grooming movement of the legs, in which the tarsal claws are scraped across the area of stimulation. Either the ipsi- or contralateral leg may be used; continued stimulation brings both legs into action. This is true of all sensilla near the midline. Towards the lateral edge of the sternum, the ipsilateral leg is always brought first into action; this agrees with the anatomical connexions described. Stimulation of each part of the sternum and accessible parts of the pleuron produces different, correctly oriented grooming responses; stimulation of the leg itself produces a movement of that leg to withdraw the stimulated area from the needle. A leg never attempts to remove a stimulus from a contralateral leg, as it does from the sternum, unless it is the inside base of the coxa which is stimulated.

Complete grooming responses are given to stimulation of a single hair, provided the displacement is considerable (cf. results of direct recording, below).

Thus a complex series of reflex arcs exist within the prothoracic segment involving input along nerve 2 and motor output along the leg nerves. The system reacts differently to different areas of stimulation, which indicates some form of spatial representation in the input along nerve 2. Similar systems must also exist around the sensory nerves from other joints of the leg.

Stimulation of spine hairs in this way always produced grooming movements, and not the type of response one would expect if the stimulation corresponded to normal proprioception. A different quality of stimulation would be expected in the latter situation, as input will be received from the hairs which engage the spine hairs as well as from the spine hairs themselves; but the fact that proprioceptive disturbances are produced by section of the commissures to the head might imply that the head ganglia are involved in complete proprioceptive responses. Certainly it can be shown by direct recording that sensory information from both spine and legs ascends to the head.

DIRECT RECORDINGS FROM NERVES

Preparation

The prothoracic sternal floor, together with a small flap of mesothoracic sternum at its posterior margin and the ventral cervical membrane at its anterior margin, was cut from the animal. In most experiments this piece was pinned to a pedestal of plasticine to allow dissection of the nerves from above, and maximum access to the sensilla of legs and sternum. As, however, nerve 2 derives from the sensilla distributed over the entire sternum, and the three proximal leg joints, this method continually excites some sensilla. For more critical work, the preparation was suspended from two horizontal Nichrome wires passing under the sternal apophyses, with only the tarsi in contact with the plasticine base.

RECORDS FROM NERVE 2

Owing to the small size of the nerve it was not possible to record with external electrodes from the larval instars or from separate branches of the adult nerve. Records were made from the nerve of adults immediately before it enters the ganglion; here it runs free for some 300μ, and can be lifted on fine hooked electrodes.

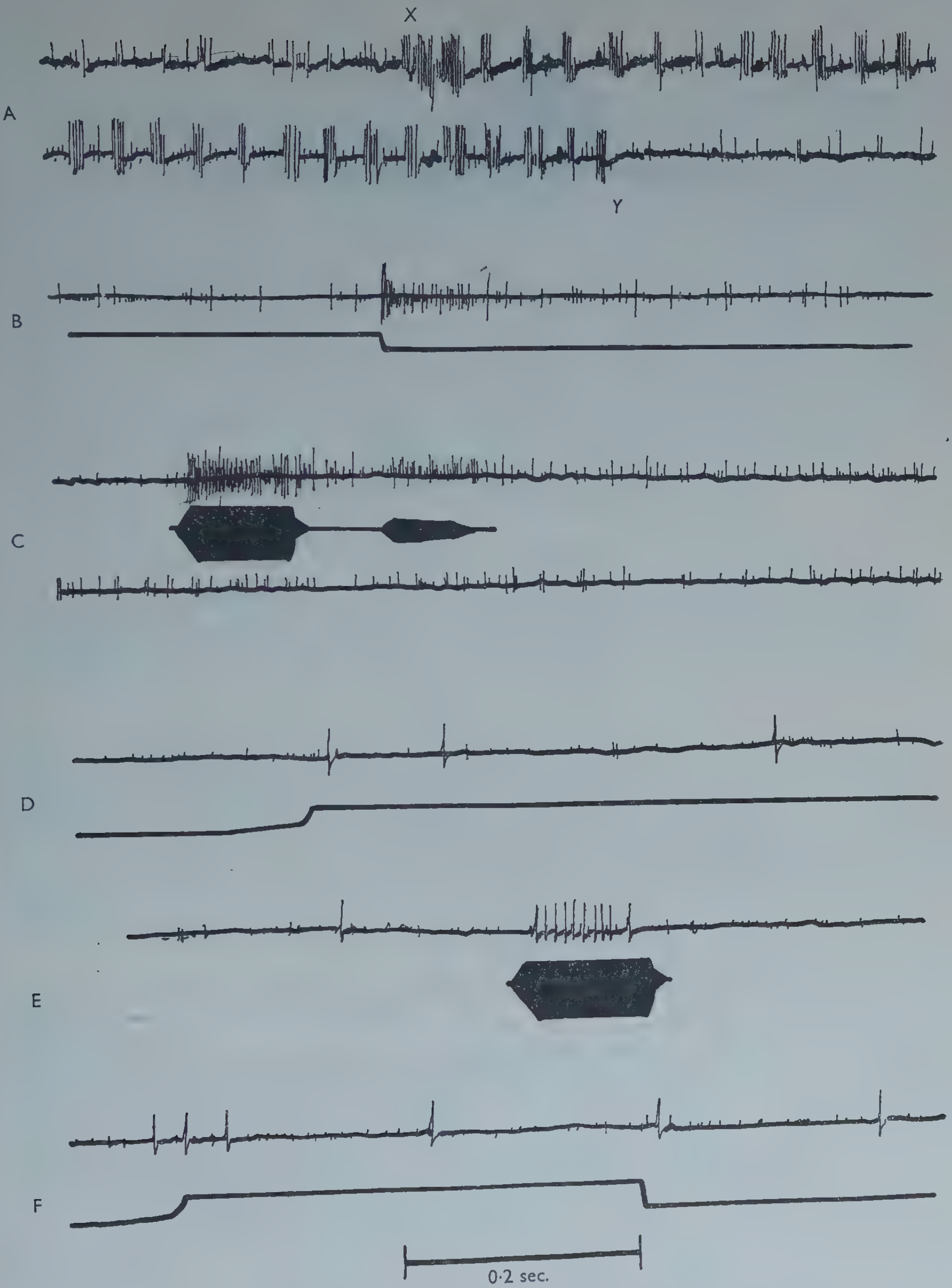
No response was detected to radiant heat, or to the vapour of xylene, benzene or pyridine, or to pure tones from 20 cyc./sec. to 10 kilocyc./sec. The hairs are probably too long and flexible to be adequately displaced by sound waves.

Slight or medium deflexion of a single hair on the spine or elsewhere produced no discernible activity. An angular deflexion of 70° or more produces an initial burst of large spikes lasting about 0·05 sec., and then there follows a rhythmic sequence of short bursts each of about five large spikes in 0·02 sec., at a constant rhythm of about 30 per second. The frequency varies from hair to hair—25 to 36 per second have been recorded—but is constant for any given hair. Under the experimental conditions, no adaptation took place during a maintained displacement of 8 min. The activity ceases immediately the hair is replaced—there is no after-discharge (Text-fig. 7, A).

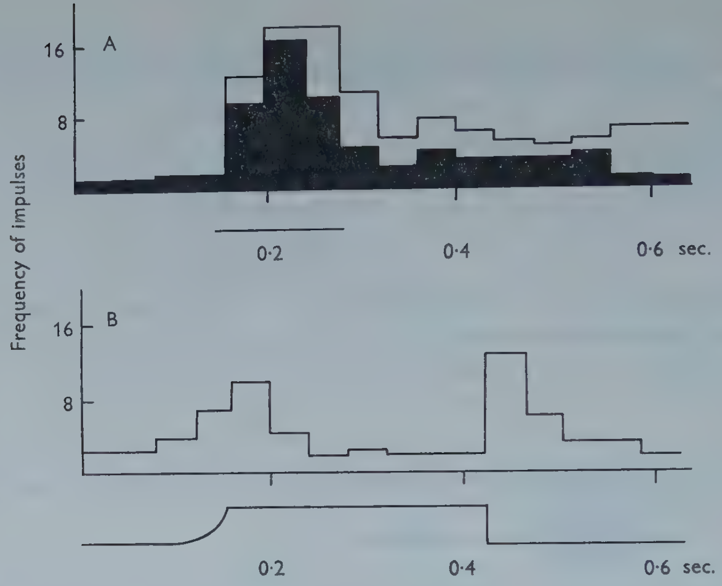
When a group of hairs, three or more, was moved simultaneously, a small discharge occurred. This was a short burst of 10–20 large spikes and a longer lasting burst of small ones, of about 1/5 the amplitude of the former. The same effect was seen on replacement. The frequency of the larger spikes depended on the rate of movement of the hairs (Text-fig. 8B). Similar results were obtained from several other groups of hairs, including both ipsi- and contra-surfaces of the spine. Raising or lowering of the flap of mesosternum relative to the preparation caused hairs present along the anterior margin of the former (which are innervated from the mesothoracic ganglion) to move some of the hairs on the prosternal spine. The activity recorded in nerve 2 resembled that obtained by moving a group of hairs with a needle as described above. A complex burst of large and small spikes occurred at both displacement and replacement; faster movement gave a higher frequency of impulses (Text-fig. 7B). The activity did not persist more than 0·2 sec. after the end of movement in either case. When some hairs of the lateral surface of the spine were stimulated by moving a thin glass rod horizontally towards the midline, simulating an observed movement of the coxa, another complex burst appeared, but of different form. Here the large spikes predominated during displacement and the small during replacement.

When the majority of the sensilla were moved synchronously, as by a puff of air, a large and complex burst was recorded. Under these conditions a very small movement of the individual hairs suffices for a complete response. The number of fibres involved increased a little with strength of stimulation, but large, medium and small spikes were always present. The system is very sensitive to change in intensity of air currents; a weak stimulus, 0·2 sec. long, gave quite a large burst but little after-discharge, while a strong one of the same length produced a burst not many times larger but with an after-discharge which was still twice the resting level 3 sec. after stimulation had ended (Text-fig. 7D, 8A).

The properties of the sternal and coxal hairs are the same as those of the spine. The sensilla of the femur are mainly of the short type, which are not easily manipulated with a needle, but they give the same response to air currents as do the larger ones.



Text-fig. 7. A. Strong displacement of a single spinal hair sensillum. Displacement at *X*, replacement at *Y*; sustained displacement of 2 sec. separates the upper and lower trace. B. Medium displacement of several hair sensilla, caused by moving the mesothorax relative to the prothorax. C. Slight displacement of many hair sensilla, caused by a double puff of air to the spine. The two traces are continuous. Note the long after-discharge. D. Medium displacement of several hair sensilla with a needle. E. Puff of air to spine. F. Slight displacement and replacement of cervical membrane relative to prothorax. A–C are recorded from nerve 2, D–F from the anterior commissure. Tracings of original records.



Text-fig. 8. Activity recorded in nerve 2 after different types of stimulation. A. A weak (shaded) and a strong (unshaded) puff of air to the sternal spine. In the latter case the activity lasts for several seconds. B. A group of hair sensilla slowly displaced and rapidly replaced.

RECORDS FROM COMMISSURES

If the sternal or coxal sensilla are stimulated and activity recorded in the anterior or posterior commissures, a great burst of impulses is recorded. These, however, are almost all correlated with the reflex movement of the legs. If the leg nerves are cut, the response in the anterior commissure is greatly reduced and that in the posterior commissure abolished. When the sensilla are covered with wax, or when the second nerves are also cut, this activity vanishes from the anterior commissure, leaving only the spontaneous activity of the ganglion.*

The results described below were obtained from ganglia which were completely de-afferented except for the second nerves.

Medium deflexion of a single hair produces no detectable activity in the commissure. The same is true of extreme deflexion of a single hair, which gives continuous rhythmic discharge in nerve 2. Bending a group of hairs, which gives complex activity in nerve 2, produces activity in a single fibre in the commissure; this fires first at the moment of maximum deflexion, and then at irregular, progressively increasing intervals of about 0·2 sec. while the displacement is maintained. No regular activity could be correlated with replacement, though this also gives a burst in nerve 2 (Text-fig. 7D).

A puff of air to spine or coxa, which produced a complex burst and a long after-discharge in nerve 2, gave a burst of a few large spikes in the commissure. The burst did not usually last longer than the stimulus, and never longer than twice the length of stimulus, although activity in nerve 2 continues for a much longer time (Text-fig. 7E).

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* While the wax used for this control is warm, a small regular discharge occurs in the anterior commissure, decreasing and vanishing as the wax reaches room temperature. Presumably some sensilla are sensitive to temperature changes of this sort, though not to radiant heat (above).

When one of the second nerves was cut, and recording made from both the ipsi- and contra-commissures after a puff of air to the spine or coxa, closely similar records were obtained. This shows that in addition to receiving information from sensilla on both sides of the animal, each nerve connects with both ipsi- and contra-commissures.

If the cervical membrane is lightly stretched forward, as in life, and then slightly displaced by a relay-operated needle, a response can regularly be recorded in the anterior commissure of fourth and fifth instar nymphs, and occasionally from adults. It is probable that this displacement is registered and transmitted by the chordotonal organs in the manner suggested above. Activity is confined to a single commissural fibre, and commences before displacement has reached its maximum (Text-fig. 7F); it appears to be very sensitive to small movements. The activity in nerve 2 during this stimulation was not successfully recorded, owing to the difficulty of dissecting far enough without damaging or stretching the chordotonal organs.

The results of these recordings are summarized in Table 1.

Table 1. *Types of activity recorded in second prothoracic nerve and in anterior commissure after various types of stimulation of sternal sensilla*

(Recordings from commissure made after complete de-afferentation of ganglion, with exception of nerve 2.)

Stimulus at sternum	Activity in nerve 2	Activity in anterior commissure
Medium deflexion of single hair	—	—
Strong deflexion of single hair	Sustained rhythmic activity	—
Deflexion of group of hairs	Complex burst	Single fibre
Puff of air to many hairs	Complex burst, long after-discharge	Single or few fibres, no after-discharge
Displacement of cervical membrane	?	Single fibre

DISCUSSION

The hair sensilla of the sternum respond to displacement caused by movement of neighbouring parts of the body, and by air currents. The sensilla of the sternal spine do not appear to differ in any way from those of the sternum, or from some of those of the leg, and it seems that the structure serves mainly to increase the amount of proprioceptive information available, by increasing the number of sensilla in the most effective area. The hairs on the spine do transmit detailed information about the relative position of neighbouring parts, and loss of this information impairs, at least temporarily, the animal's control over them.

It is not clear why the Catantopinae in particular should supplement their proprioceptive input in this way; most members of the family are active and manipulative, but not conspicuously more so than, say, the Oedipodinae, which produce convergent forms in most habitats. Certainly within the Catantopinae there seems to be a correlation between a sternal spine well supplied with sensilla and manipulative ability of the forelegs. *Schistocerca* is an active animal, travelling from one food plant to another, picking food up and inspecting it when it is found, conveying small pieces of leaf to the mouth, turning them edgewise to go between the mandibles; in captivity it will rake together with the forelegs pieces of bran from a rather watery gruel, and then lift them to the mouth. *Ornithacris*, a much larger, more sluggish animal, lives in reed-beds. The leaves it prefers are too large to be handled, and it often merely gnaws thick stems. The spine is well developed, but with comparatively few sensilla. *Romalea*, yet more sedentary, has hardly any sensilla in the sternal region.

The interest of the system described lies chiefly in the organization of the nervous system which handles the tactile information. Two levels of integration of the signal from the sensillum are seen—at the nerve fibre and at the ganglion. It is common in insects for several sensilla to be served by one sensory nerve fibre, and this is well seen in the present material. At all but the highest levels of stimulation, it seems that more than one sensillum must be excited before a propagated impulse occurs in the nerve. Only when a single sensillum is stressed to the maximum does it pass a signal to the ganglion (perhaps as a warning signal to avoid damage?). If the area of excitation includes sensilla connected to different nerve fibres, and the properties and/or connexions of the nerve fibres differ, then the system offers an economical method of distinguishing spatial patterns of stimulation. These properties are present here in the considerable variation in diameter of fibres all deriving from similar sensilla, and in the very different types of activity produced by stimulating different groups of hairs.

Of all the complex information which arrives at the ganglion through the second nerves, a strikingly small proportion, and that greatly simplified, is transmitted to the brain. Only when a number of sensilla are excited simultaneously, as by air currents, is activity produced in one or very few fibres of the commissure. Apparently the majority of decisions are made at a segmental level, and this is confirmed by the complexity of the segmental reflexes which stimulation produces. The performance of the reflex, however, results in a great deal of activity in the commissure; it is to be expected that this information about the movement of effectors would be necessary to the highest co-ordinating systems, which are cerebral.

SUMMARY

1. The prothoracic sternal spine of *Schistocerca* is described. The second paired nerve innervates the sensilla of the sternum, coxae, and femora, and in subimaginal stages supplies chordotonal organs registering movement of the cervical membrane. Each nerve fibre serves the axons of several sensilla.

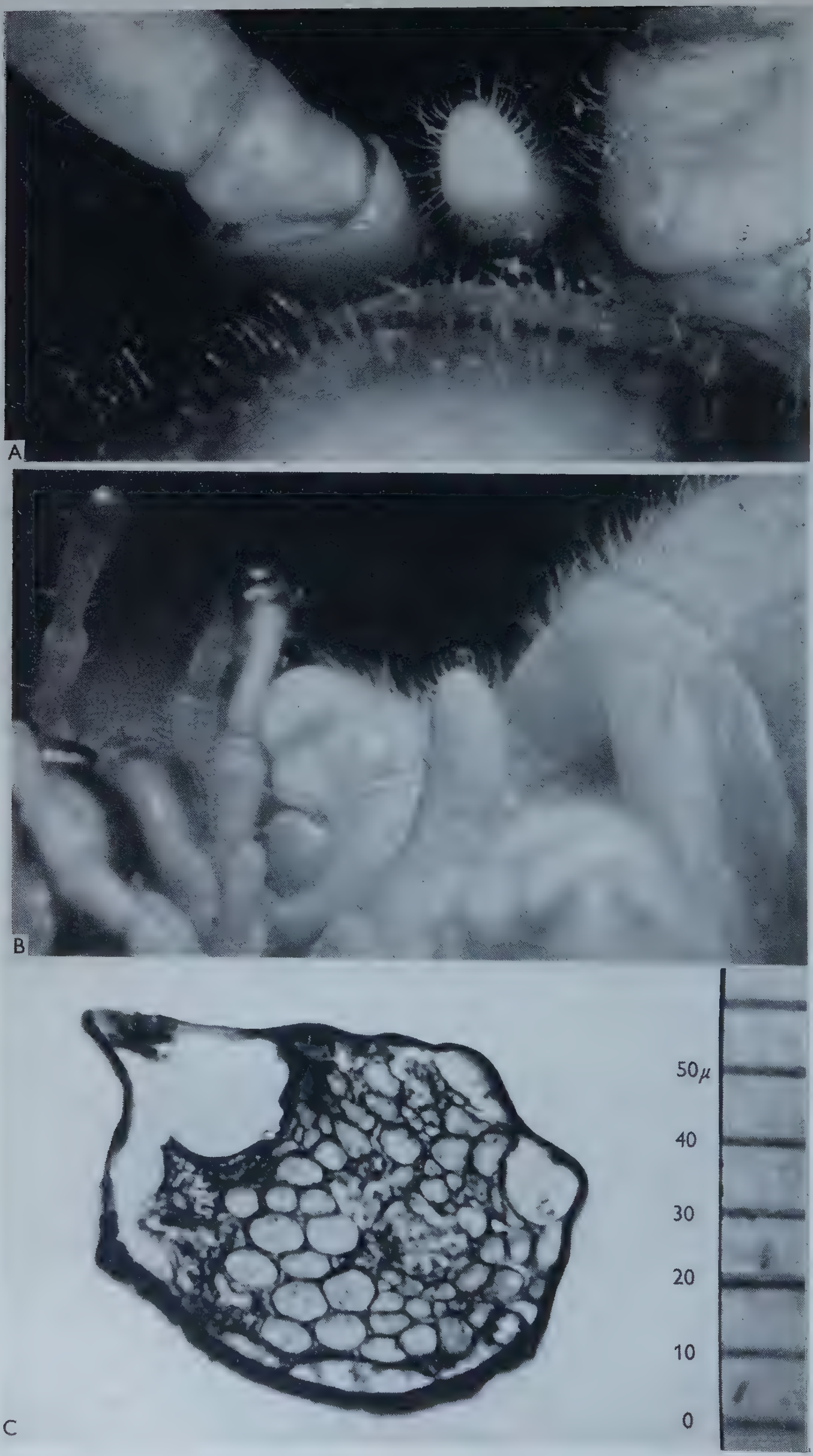
2. When the sensilla are waxed, control of the legs is disturbed.

3. When the prothoracic ganglion is isolated from the CNS stimulation of the sensilla produces complex, oriented, reflex cleaning movements by the legs.

4. Recordings were made from nerve 2 and from the commissures. Co-ordination is mainly segmental; the signal in the commissures is much reduced. At normal levels of stimulation there is no activity in nerve 2 unless several sensilla are excited.

5. Among related genera there is some correlation between manipulative ability and the number of sensilla on the spine. It is suggested that these sensilla contribute to the co-ordination of movements of the front legs.

This work was undertaken during the tenure of a D.S.I.R. Research Fellowship.



C. H. FRASER ROWELL

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EXPLANATION OF PLATE

A and B. The sternal spine of *Schistocerca*, showing the profusion of sensilla, and the way that those located on different parts contact each other.

C. Transverse section of nerve 2, near ganglion. Picric-osmium/haematoxylin.

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THE CENTRAL NERVOUS CONTROL OF FLIGHT IN A LOCUST

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INTRODUCTION

That proprioceptive feedback is important in the regulation of locomotory patterns has been widely demonstrated. In fact, reflexes based on such peripheral loops have been suggested as adequate to produce the pattern and rhythm of movements in arthropod walking and flight. The present study characterizes some of the sensory input during flight of the locust which could be expected to be involved in these reflexes. However, it will be shown that this input is unnecessary for the establishment of a patterned oscillation in the thoracic ganglia which appears to be the same as that which produces the flight movements. The feedback from the moving parts of the flight system modifies considerably the frequency and details of ordering of a pattern which, nevertheless, is inherent in the ganglia. The elicitation and maintenance of this pattern depends on some source of excitatory input which need not be phased to the flight movements.

The desert locust *Schistocerca gregaria* Forskål is a particularly favourable object for such studies for a number of reasons. It can be induced to fly in the laboratory for extended periods of time with almost constant, cyclical and strictly co-ordinated movements of its two pairs of wings (Weis-Fogh, 1956*a*). The fundamental oscillation of each wing is up and down, but superimposed upon this is an active twisting of the wing surface, the pronation, starting at the beginning of the downstroke. The reverse supination is mainly passive-elastic and prevails during the upstroke. Each wing is moved up by the contraction of a medial row of vertical elevators and moved down partly by elasticity (Weis-Fogh, 1959), partly by a dorsal longitudinal depressor muscle and, finally, by a lateral row of vertical depressor muscles which also control the twisting (two basalar and one subalar on each side in each segment). Each of these muscles consists of one to four fast-type motor units which are activated zero, one, or two times per wing stroke (Weis-Fogh & Wilson, 1961). The typical motor output then consists of a rather simple score of nerve impulses strictly correlated in time with the observed wing movements and repeated at the same frequency (Weis-Fogh & Wilson, 1961). It is this remarkable quantitative reduction in number of units and activities of the wing system that makes it worthy of study.

The studies of Weis-Fogh (1956*b*) on sensory mechanisms in locust flight and the hypothesis on the role of these in the production of the motor command formed the basis for the experiments described here. Since the behavioural experiments showed the necessity of some control inputs and the temperature independence of the frequency of output suggested a mechanical timing step, it seemed possible that these were both necessary and sufficient for the operation of the flight control system. However, the results of the search for such all-important inputs were negative; the approach was therefore reversed and an attempt was made to make the system operate without input from the moving parts.

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Anatomy. The musculature and pertinent aspects of locust physiology are described elsewhere (Weis-Fogh & Wilson, 1961). The thoracic nervous system is described by Ewer (1953, 1954) and his nomenclature is used here. Fig. 1 depicts the main structures as they appear in a dissection similar to that of some of the preparations. The thoracic ganglia are not fused but are connected both by the main cords and more lateral recurrent nerves. The first two pairs of peripheral nerves of the pterothoracic segments are concerned in basic flight mechanisms. Nerve I connects to the recurrent nerve of the next anterior segment. Its anterior (I*A*) and posterior branches (I*Bb*) are wing sensory nerves and the middle branch (I*Ba*), innervates the dorsal longitudinal musculature. The second pair of nerves innervates the vertical flight muscles.

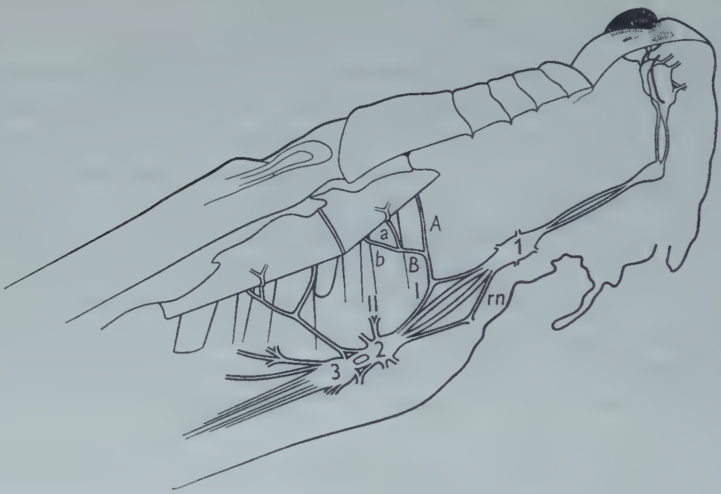

Fig. 1. Diagram of a dissection of *Schistocerca*. The dorsal tegmentary nerve supplying the head hairs is indicated as a solid line. The nerves and ganglia are numbered as in Ewer (1953, 1954). *rn* indicates recurrent nerve. The positions of the dorsal longitudinal muscles and elevator muscles are indicated. The controller depressors lie behind the elevators in this view. Many non-flight structures are omitted.

Wing movements. The wing-stroke frequency is about 17 cyc./sec. and is nearly independent of temperature over the normal flight range. The upstroke occupies about 41% of the cycle in standard flight. The two segments are 6–8 msec. out of synchrony with the hindwings leading. In general terms the contractions must follow the sequence: metathoracic elevators, mesothoracic elevators, metathoracic depressors and controller depressors, mesothoracic depressors and controller depressors, metathoracic elevators, ...

Sensory inputs. The behavioural evidence on the roles of various input sources has been summarized elsewhere (Weis-Fogh, 1956*b*). Wind-sensitive hairs on the head discharge into the cord (Haskell, 1958) and can initiate and maintain flight (Weis-Fogh, 1956*b*). Sensation from wind on the moving wings can maintain flight and also must be involved in some control measures including the control of lift against imposed changes in pitch. Loss of tarsal contact and pinching the abdomen may start flight but are insufficient to maintain it.

MATERIALS AND METHODS

Adults of *Schistocerca gregaria* Forskål, phasis *gregaria* or *transiens* were used in all experiments. These were bred at the Anti-Locust Research Centre, London. Large females at least 1 week past last moult, but not yet egg-laden were chosen for most operations. Smaller males were sometimes used with the easier techniques.

Experiments with flying animals made use of a wind tunnel and several animal suspensions already described by Weis-Fogh (1956*a*), including an aerodynamic balance. In most experiments the animal was *tethered*; that is, could not choose its own wind speed. In cases where a servo-mechanism was used to adjust wind speed to the thrust of the animal the term *suspended flight* will be used.

For electrical recordings contact with the animal was via $40\,\mu$ or $100\,\mu$ platinum or platinum-iridium wires, either bare or insulated to near the tip. All recordings were extracellular. Both mono- and bipolar recording technique was used dependent upon each special case. Tektronix type 122 preamplifiers and a Tektronix type 502 oscilloscope were used to amplify and display electric signals. Films of the oscilloscope were taken with a Shackman type no. AC 2/25 Oscilloscope Recording Camera. Electrical stimulation was accomplished with a two channel Disa Multistim. For repeated trains of stimuli the Multistim was driven by the synchronizing output of a stroboscope, General Radio Corp. Strobotac, also used for visual observations of wing and muscle movements.

Operations from which recovery was necessary, such as cutting of sensory nerves and central connectives, were carried out after CO_2 anaesthesia. Effort was made to reduce locomotory and respiratory movements without abolishing the latter. For longer operations a mixture of CO_2 and O_2 gases was used.

Acute preparations were made without anaesthesia. In highly dissected preparations some of the damage to the respiratory system was compensated by aerating the main tracheal supply to the central nervous system. This was accomplished by introducing moistened air at a pressure not greater than 20 cm. of H_2O through a cannula inserted into the mandible. Air in one mandibular air sac reached both longitudinal ventral trunks. Escape of air from each longitudinal ventral trunk cut posterior to the thoracic nervous system was ascertained by watching bubbling of air or eddying of liquid surfaces near the cut ends. This flow of air was found necessary for the maintenance of the nervous responses to be described.

The locust saline of Weis-Fogh (1956*c*) was used saturated with air but without penicillin. In those experiments requiring saline solution, damage to the nerve sheath was either peripheral or at some distance along the cord from the pterothoracic ganglia and the ganglia under consideration were probably not exposed to any unusual ionic medium.

The temperature of the room during all experiments was about 25–27° C.

RESULTS

I. *Sections of the nerve cord and recurrent nerves*

Destruction of the supra-oesophageal ganglia or removal of the abdominal nervous system had the same effects as previously reported (Weis-Fogh, 1956*b*). In good preparations normal flight occurred. Complete decapitation, removing the sub-oesophageal ganglia, resulted in an animal which could give an apparently normal tethered performance. The same effect was produced in another animal by a careful operation in which the connectives were cut in front of thoracic ganglion 1 and the wound sealed with wax. On the next day no response could be obtained to wind on head. The animal could walk and jump well. Once started to fly by tarsal reflex it could generate lift greater than body weight and maintain flight in wind. Post-mortem examination showed the connectives to be completely severed. The basic co-ordinating system for flight must be independent of these higher centres.

Results of cuts between thoracic ganglia 1 and 2 on four animals are not so clear. In some cases control operations in which the cord was exposed but not cut resulted in weak fliers. In all four animals some flight movements could be elicited by pinching the abdomen or by tarsal reflex. These were usually only weak flutterings of the wings. The best result was obtained in one animal by stimulating the abdominal cord electrically. This resulted in somewhat low-amplitude wing beating at 13–14/sec. The phasing between fore- and hindwings was normal and wing twisting occurred as usual. The flight continued in wind for a few minutes after a long stimulation. The recurrent nerves were cut along with the connectives in these operations. The success of the operations was checked by observing the walking pattern. The forelegs were no longer co-ordinated with the hindlegs and stimuli on either side of the cut caused the legs in that side only to respond. Post-mortem examinations were also confirmatory.

Section of the connectives and recurrent nerves between thoracic ganglia 2 and 3 in three animals gave the expected effects on leg reflexes. Animals walked with the front two pairs, dragging the third. Ten Cate (1936) reports co-ordinated movements after such a cut in *Locusta*. This is true only to a slight extent in *Schistocerca*. A hindleg will occasionally step when it is dragged. Pinching the abdomen gave good jumping responses. Blowing on the head of a suspended animal resulted in flight posture of the forelegs and flapping or weak trembling of the forewings. Often the forewings could not unfold but if placed open would flap. The amplitude was low and in the one case measurable the frequency was about 12/sec. Wing twisting did not occur. Probably only a few muscles were active and not the main pronators. Perhaps only the elevators were active and downstroke was due only to elasticity. It will be shown later that the mesothoracic depressors are not exclusively excited through the third ganglion since they may be caused to oscillate with the elevators even when the metathorax is quiet if the anterior cord is stimulated electrically. It is not yet possible to decide whether or not the main excitation of the mesothorax comes from the metathoracic ganglion. In the same preparations the hindwings flapped weakly when the abdomen was pinched. There appeared to be some coupling between the wing pairs. When either pair was flapping some very weak twitching movements were noticeable in the other. These were not entirely passive mechanical movements since they were not always present, and therefore probably indicate reflex activation of some muscles in the adjacent segment.

In one animal only the recurrent nerves between 2 and 3 were cut. The post-mortem dissection gave a clear check on this. In a wind of 2·5 m./sec. the wings moved in proper phasing, twisted a little weakly, with wing beats of rather low amplitude and frequency of about 13/sec. Blocking the wind to the head did not stop flight, but did lower the frequency a little. This damage seems to have weakened the flight but not to have reduced co-ordination. If the effect is not just a general one due to the operation itself, it would seem to indicate a sensory disturbance rather than a co-ordinative one.

During recovery from operations between ganglia 2 and 3 spontaneous twitchings in the wings and legs were seen for an hour or two. Muscle recordings from these operated animals will be described in a later section.

The main flight-control pattern is established in the pterothorax with possibly some necessity for the prothoracic ganglion. Subdivision of the thoracic nervous system results in partial flight patterns in which the number of components active and the frequency of operation are reduced.

II. *Recordings from sensory nerves*

Sensory recordings were made in three types of preparations representing differing degrees of dissection. In the least dissected animals the legs, the pronotal shield, and the last two segments of the abdomen were amputated. The soft cuticle between the prothorax and pterothorax was cut except ventrally. The gut was pulled out through this anterior hole. The thoracic air sacs were cleaned away and electrodes fixed to the cuticle so that they extended into the half-empty thorax. A nerve was then lifted over the electrodes and thereby suspended in the moist air of the thoracic cavity. The anterior part of the body was waxed back into place so that wind did not enter the cavity and air-stream stimulation would be near normal. These preparations gave good tethered performances for at least 1 hr. One animal treated similarly, except for placing electrodes, was released by throwing it into the air. It flew away in level flight indicating that the structures removed, including much of the sympathetic nervous system, are not necessary for controlled flight and that this amount of general damage to the animal does not destroy flight reactions.

In a second recording situation the isolated pterothorax was mounted and electrodes brought into the thoracic cavity and placed under various nerves. The wings were moved from the elastic equilibrium position passively by pushing down on the notum or pulling up on the wings with attached threads or actively by stimulating muscles electrically.

Other recordings were from sagittally split thoraces in which the central nervous system was removed. In these it was easier to pick up the nerve desired. Confusion due to reflex effects and motor discharge was not possible. These preparations were also mounted so that the wings could be moved through their normal range.

The anterior branch of the first pair of nerves in each flight segment (nerve 1*A*) is apparently purely sensory and innervates the wing and the anterior two-thirds of the wing-base cuticle. Included in it are fibres responding to tactile stimulation of the wing surface. Sotavalta (1954) has reported these in a lepidopteran. If the wing is cauterized near the base these responses disappear, but there are still present numerous units responding to wing movements, especially downward movements. It is not unreasonable to suppose that these fibres innervate the campaniform sensilla on the basal regions of the large veins, since in the forewing at least no other route has been found over which these may connect to the ganglia. Organs in the wing hinge may also contribute to the discharge in this nerve, but no evidence is available on this.

The in-flight discharge through nerve I*A* of the forewings (mesothorax) is shown in Fig. 2. The number of responsive units cannot be counted. Most of the response occurs during the middle of the downstroke. The conduction distance from the sense organs is about 4–5 mm. The fibres can be judged from the amplitude of the recorded response to be much smaller than the motor fibres in nerve I*Ba* (to the dorsal longitudinal depressor) which conduct at about 3 m./sec. From an estimation of probable velocity of 0·5–1·5 m./sec. it is expected that the main response in nerve I*A* begins near to top wing position and is maximal during mid downstroke.

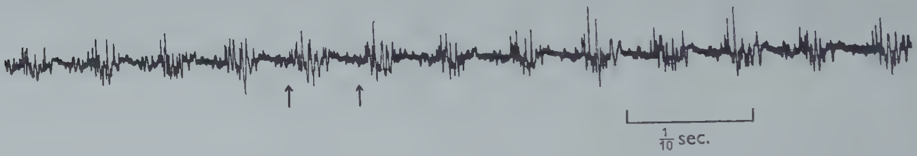

Fig. 2. Sensory discharge in mesothoracic nerve I*A* during flight. The arrows indicate the approximate time of the top of the forewing stroke.

The posterior branch of nerve I (nerve I*Bb*), according to Ewer (1953), has its main ending(s) on the soft cuticle just below the subalare. Electrical recordings from this nerve in the mesothorax show one large unit together with a few relatively very small ones. The recorded amplitudes of the smaller spikes were only a few per cent (under 10%) of the amplitude of the conspicuous unit. The small units responded to touching and moving the wing, but the response to movement was not orderly.

The single large unit in the mesothoracic nerve I*Bb* gave a proprioceptive response which was some function of wing position, rate of movement, and direction of movement. In the wing-down or folded position the unit was spontaneous at a low frequency, at most a few times per second. This rate depended on activity minutes before. After a period of flight or manipulation of the wing the unit was often silent for a minute or two. When the wing was elevated either muscularly, or by pushing down on the notum, or by pulling directly on the wing, the frequency increased and adapted to a new higher level dependent upon the degree of elevation. In a high position the discharge might remain at about 40/sec. for minutes. During upward movement the frequency was higher, up to 350/sec. for an extreme pull. Downward movement abolished all response. When downward movement was stopped the tonic frequency for that position gradually returned. The recorded frequencies at different positions varied from animal to animal but the qualitative relationships were always the same and this unit could be identified in every preparation.

The discharge of this unit during flight, is, of course, phasic, but it is possible that some averaging of tonic effect over several cycles might influence the frequency of the phasic response. During tethered flight the unit fires one to several times toward the end of upstroke with occasional firings just after the up-position. Fig. 3*a* shows this response in a preparation in which the central connexion of nerve I*B* is cut, leaving the dorsal longitudinal muscle of that side denervated, but eliminating the possibility of confusion with motor impulses. Fig. 3*b* shows the response with the nerve intact. The upper trace is a record of muscle action potentials in the first basalar muscle which has only one motor unit. A variable elevator muscle unit potential shows up in the lower trace. The large mainly downward spikes are motor impulses to the dorsal longitudinal muscle. The smaller mainly upward spikes are from the sense organ. If a few milliseconds are allowed for conduction, the record indicates firing of the sensory unit mostly just before activity in the basalar muscle which fires 2–3 msec. before the top position (Weis-Fogh & Wilson). In Fig. 3*b* a correlation is seen between number of firings of the sensory unit and activity in one elevator muscle. Activity in the sensory unit is greatest at the beginning and end of flight (Fig. 4). The records show also that elevator muscle activity predominates over depressor activity these times. The time relationships indicate that the increased activity in the elevators causes more sensory firings and not vice versa. An effect of the increased sensory firings is not discernible in the records even though three differently acting muscles can be observed and frequency and other time relationships measured. If there is a discrete cycle-by-cycle effect it remains obscured.

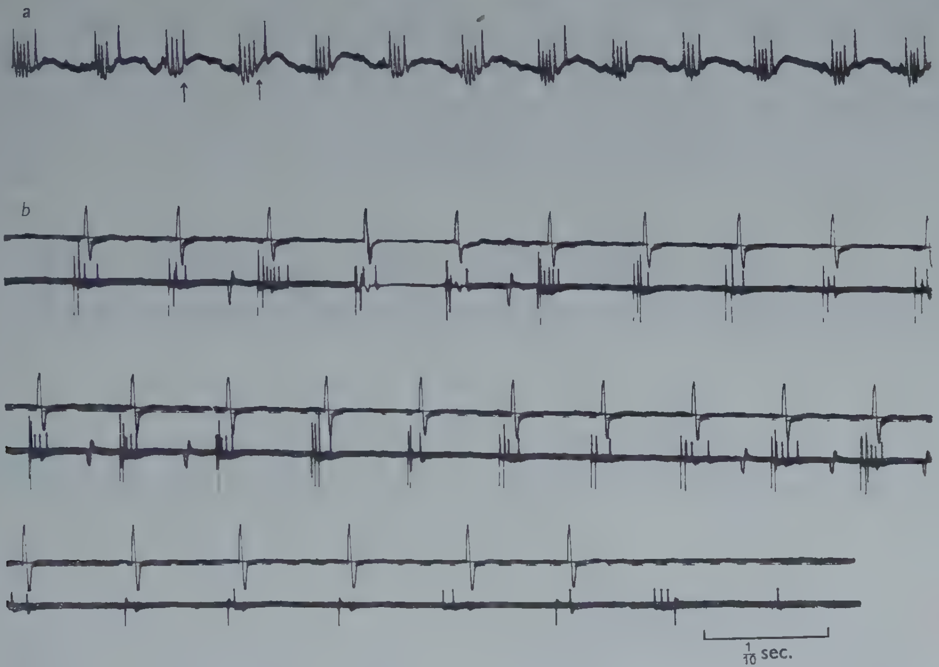

Fig. 3. (*a*) Sensory discharge in mesothoracic nerve I*Bb* during flight. The arrows indicate the approximate time of the top of the forewing stroke. (*b*) Upper trace, electrical record from the first basalar muscle of the mesothorax. Lower trace, sensory and motor discharge in mesothoracic nerve I*B* and action potential from one elevator muscle unit. Nerve spikes beginning downward are motor. The smaller mainly upward spike is the sensory unit. The three strips are from parts of the same flight, the last showing cessation of flight.

Recordings from the nerves of the hindwing were made only in non-flying preparations. The results were similar except that the metathoracic nerve I*Bb* contains some additional large units including one which responded in a way opposite to that above, in other words a *down* unit.

Discharge due to wind on the head has been recorded from the whole cord (Haskell, 1958). This discharge consists of many asynchronous units which adapt quickly to a maintained level. The discharge appears continuous rather than temporally patterned. There is certainly no large burst formation at a frequency near that of flight. Single units have not been followed.

In summary, there are both tonic and phasic inputs to the thoracic central nervous system during flight. These have at least the potential capacity for indicating wing position and magnitude of wing movement. The behavioural evidence shows that wing forces must be measured as well. Presumably sufficient information is carried centrally to allow of a pure reflex integration of the flight pattern.

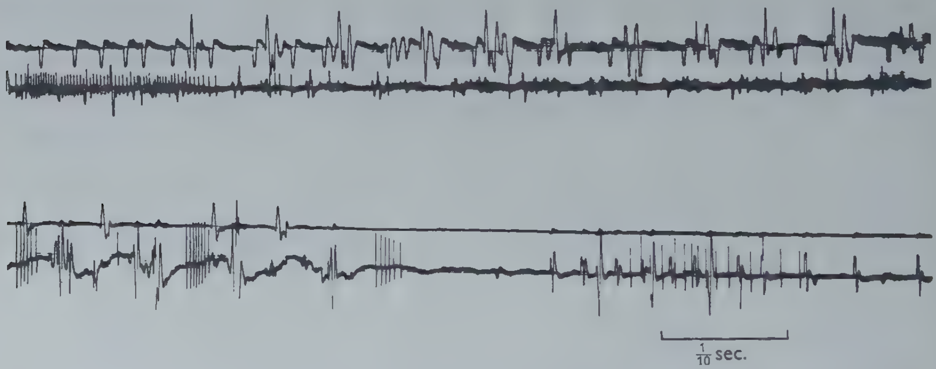

Fig. 4. Upper trace, muscle action-potentials from an elevator muscle (downward) and the overlying first basalar muscle of the mesothorax. Lower trace, record from mesothoracic nerve I*Bb* plus some elevator muscle activity. The first strip shows the beginning of flight, the second the end, including an extra burst. Vertical amplitude was increased in the lower trace of the second strip.

III. *Sensory reduction*

The various sources of input described were eliminated operatively in an attempt to determine their precise roles in flight control. It was shown by Weis-Fogh (1956*b*) that the wind-sensitive exteroceptors on the head are sufficient to initiate and maintain flight at least when the thoracic proprioceptors are intact, but that if the wings move in wind, head input is not necessary. In the following experiments the head hairs were stimulated by wind except in specifically stated cases.

A. Whole wings or portions of wings may be amputated in various combinations without changing the basic pattern of wing movements. Slight changes in the over-all frequency and in amplitude of beat of the remaining members may occur, but a simple regularity of response has not been detected in the few dozen cases tested. Decreasing the length of all four wings does not result in increased frequency as it does in the myogenically controlled insect flight systems. There is a tendency rather to decreased frequency. If the wings are all cut off as near as possible to their bases and the frequency measured by stroboscopic observation of the nota or wing stumps it is found that the rhythm is no longer smooth but changes often and quickly over a rather wide range. The measurement is consequently difficult, but values from 13 to 20/sec. certainly occur in the same animal within a few minutes. In some cases it is necessary to increase the wind velocity to unnatural values in order to keep the flight going. The decreased input due to the amputation can apparently be compensated in some ways by increased input via another route. The unsteadiness of the frequency of response may be due to near-threshold condition of a few important units.

B. A branch of nerve I*A* enters the tegula. In three animals all four tegulae were severely cauterized. Destruction of sense endings therein should have been quite complete. All three animals still flew in a flight balance with lifts from 70 to 90% of body weight and at wing-beat frequencies of 16–18/sec. at first, with both lift and frequency gradually decreasing. One animal was thrown high in the air. It flew nearly straight, in good balance, but slowly lost altitude. No specific damage to the flight system is indicated in this set of experiments although the performances were a little weak.

C. On seven animals an attempt was made to cut the whole of each nerve I*A* of meso- and metathorax. This was done by removing the soft membrane surrounding the tegula, locating the nerve and lifting it with forceps, and cutting as far centrally as possible (*ca.* 2 mm. ventral to wing hinge). Tests of the success of the operation were of a negative kind. In one case electrical recording was attempted on a dissected animal. No response was found in the nerve with large passive wing movements. Dissection was twice attempted to check for continuity of the nerves, also with negative results. It is possible, however, that some fine twigs have remained intact in some preparations, but this is highly unlikely for them all. The results were fairly similar in all animals at the beginning. The lasting qualities of the preparations were more variable, with some deteriorating rapidly. In all these animals the posture, wing twisting, and wing phasing appeared normal. All gave frequencies between 15 and 17/sec. at first, going down to minima of 13–15/sec. Lift was measured in three. Before the operation this was equal to or greater than body weight in each, but later was only one-half to three-quarters body weight in long runs. For short bursts it was higher, as when starting or when stimulated with light flashes. Preferred wind velocities were under 3·0 m./sec.

The head hairs of three of these animals were painted with celluloselac. After this dried no response was obtained with air-puff stimuli. One animal now could fly for only a few tenths of a second when started by loss of tarsal contact in wind. Another maintained flight for a few minutes at a time. The third flew for long periods in a wind of 3 m./sec. However, it would not fly with a speed greater than 2·5 m./sec., so free flight would have stopped. The effect was reversed by removal of the paint.

Again no specific flight damage can be pointed to. The animals flew quite weakly but comparably to ones in which other types of operation have been made not involving sensory reduction in the flight system. The nerves I*A* may play a role in maintenance, but not an exclusive one. They have at most a small function in frequency control. They could be involved in lift control, but this was not studied.

D. More striking results were obtained when each entire nerve I was cut. In order to do this the abdomen was removed, the prothorax loosened except ventrally, and the gut taken out. The five animals could still fly when tethered in wind. The frequency before nerve section was at the low end of the normal range. After nerve section the frequency dropped and the rhythm became a little unsteady, making stroboscopic observations difficult. The dorsal longitudinal muscles are, of course, denervated in this operation and the downstroke is due to elastic forces plus the activity of the controller depressor muscles. Electrical recordings of muscle action-potentials were combined with approximate stroboscopic frequency values. Three values were 8, 9, and 11/sec.; the other two were crudely estimated as at about one-half normal frequency. The flights were of short duration. The decrease in frequency is probably not due only to inactivation of the dorsal longitudinal muscles. In another type of preparation described in the first paragraph of § II recordings from the motor nerve to one anterior dorsal longitudinal muscle show that it can become completely silent without the flight frequency being abnormally reduced (Fig. 5). The damage to that animal was not of the sort that would cause one to expect that only that dorsal longitudinal muscle was silent. The wing movements were approximately symmetrical. Even if it were mainly lack of activity of the dorsal longitudinal muscles which results in lowered frequencies this effect would depend on lack of, or later, sensory input. It is probable that the information for maintenance of the normal frequency does originate in the movement of the wing and is carried in nerve I. No evidence suggests that the flight muscles contain proprioceptors. Nerve I*A* does not carry any specific frequency-setting information. The large unit of nerve I*Bb* of the mesothorax could over several cycles have an influence but it hardly does so in the period of one or two cycles (Fig. 3*b*). The unanalysed units of the metathoracic nerve I*Bb* remain as possible cycle-by-cycle frequency setters.

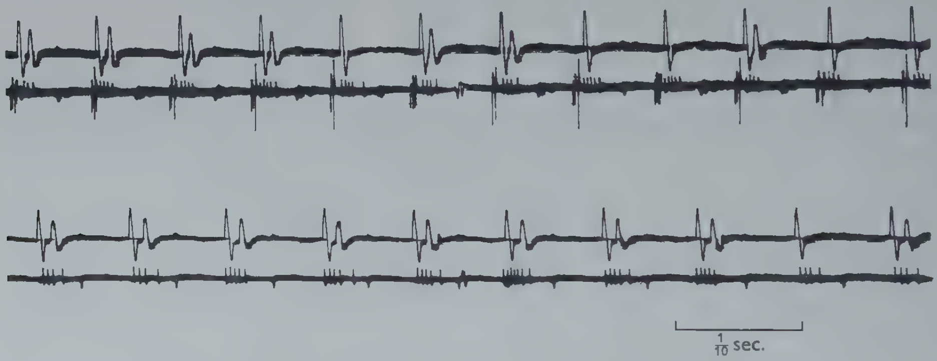

Fig. 5. Recordings the same as in Fig. 3*b*. In the second strip the motor axons to the dorsal longitudinal muscle are silent. The strips are from the same flight.

Eliminating sensation from the wing and motor innervation of the dorsal longitudinal muscles has the definite effect of lowering the frequency of the wing-beat cycle. No measurements have been possible yet on the effect this produces on the ability to make control adjustments. This reduction of input did not, however, upset the basic pattern of wing movements including wing twisting and segmental phase differences. This surprising result led to the hypothesis of a built-in central pattern which is not dependent upon peripheral feedback loops for its basic operation, but which is modified by such input. This input apparently increases frequency as well as affecting small changes in pattern which control flight.

IV. *Semi-isolated nervous preparations*

A series of experiments was carried out in which the possibilities for feedback were further and further reduced, the final form consisting only of head, thoracic nerve cord with tracheae, and a ventral cuticular strip. More than forty diverse preparations were made. Three were of the last type.

All the operations had some features in common. The wings and legs were removed, the last few segments of the abdomen cut off and the prothoracic–mesothoracic junction opened dorsally and laterally. The gut was then pulled out forward. The body was waxed into a Perspex chamber with the head projecting through a hole. The ventral tracheae were ventilated. The lateral half or the dorsal half of the thorax and abdomen were removed. The whole preparation was put at the opening of a wind tunnel and electrodes were placed as necessary. The chamber was covered with moist paper or sealed with cellophane and grease. Good preparations remained responsive for 1 or 2 hr. at 27–28° C. If placed in a refrigerator they could be kept overnight but then deteriorated rapidly when warmed up.

On nearly any locust in 'flight condition' the following observation can be made. The animal is quickly cut down to head and ventral half of thorax with the gut removed. Here all the wing sensory nerves are cut, the dorsal longitudinal muscles are removed and all other flight muscles bisected transversely. When the head is placed in wind it can be seen directly that these muscles contract in a rhythmic, patterned way, the cut elevators and depressors resembling reciprocating pistons, and opposite sides in phase. The fact that such bisected muscle stumps lengthen passively and rapidly after each contraction is unusual and is probably due to the strong passive-elastic system present in locust flight-muscle (Buchthal, Weis-Fogh & Rosenfalck, 1957). The frequency is obviously lower than normal flight frequency.

Recordings of muscle action-potentials showed the frequencies of eleven more careful preparations to be between 4/sec. and 12/sec., the mode being about 7/sec.

The frequency of cycling was measured in one lateral half-animal before and after cutting the wing sensory nerves of that side. The elimination of input from the other side and the derangement of the mechanical conditions for the intact side resulted in an immediate decrease in frequency to about 7/sec. Cutting the four sensory nerve branches caused an extra small drop to about 6/sec.

In three of the best preparations the latencies from elevator response to adjacent controller-depressor response were, 30, 30 and 40 msec. (Fig. 6*a*). The elevator-to-depressor interval was 25–35 % of the whole cycle, compared to 41 % for upstroke in the intact animal, measured from observations of the moving wings. (In intact animals the elevators and vertical depressors normally fire a few milliseconds prior to the bottom and top of the wing stroke respectively (Weis-Fogh & Wilson, 1961).) In once case the interval was 40 % of the whole cycle at 7/sec. but the same preparation shifted slowly to a less even distribution. Most of the decrease in frequency is due to increased depressor-to-elevator interval. The intersegmental lag was always small. The difference between apparently homologous elevators was up to 10 msec. with the metathoracic one leading, i.e. much as in a normal animal. Fig. 6*b* shows a typical case.

In three lateral half-animals the time relationships between the dorsal longitudinal muscles were observed with all wing sensory nerves cut. In each case the phasing varied over long periods so that first one then the other led by up to 10 msec. The phasing was constant, however, for hundreds of cycles at a time. Under some conditions, one being excessive wind speed, the mesothoracic dorsal longitudinal muscle sometimes fired excessively often and irregularly while the metathoracic dorsal longitudinal muscle continued a neat rhythm.

In three preparations lacking any thoracic muscles and with the abdomen removed recordings were made from the motor nerves. (Similar recordings were also made in preparations still having flight muscles. These have no extra interest except that they show definitely that the nerve potentials recorded are related to activity of the flight muscles.) Nerve I contains motor axons to the dorsal longitudinal muscles and apparently no others. The recordings show the same results as found in the above muscle recordings. A small burst of spikes was repeated rhythmically at frequencies between 4 and 10/sec. in different preparations. The two segments were sometimes in phase or either one led by up to 10 msec., this condition changing slowly after hundreds of cycles. In one animal the mesothoracic nerve I was higher in frequency and erratic while the metathoracic nerve I appeared normal (see Fig. 7).

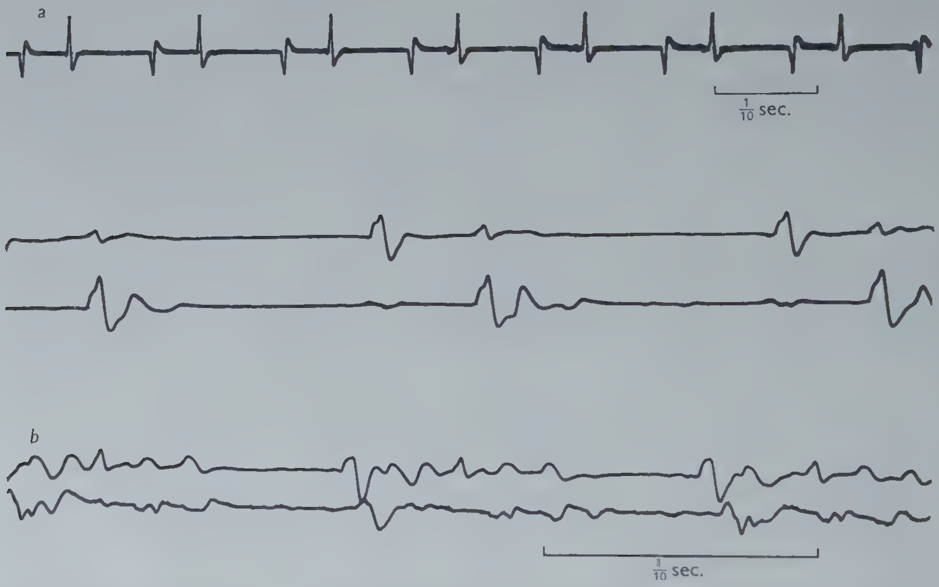

Fig. 6. (*a*) Muscle action-potentials from adjacent elevator and depressor muscles of a head and ventral half-thorax preparation. First strip: elevator spikes downward, depressor upward. Second strip: upper trace, elevator muscle; lower trace, depressor muscle. (*b*) Action potentials from elevator muscles of the same preparation. Upper trace, metathoracic muscles. Lower trace, mesothoracic muscles.

Recordings from nerve II were not so clear, there being many more units, both elevator and depressor. They do, however, show bursts of spikes repeating rhythmically at 5–10/sec. The single long burst seemed to contain both the elevator and depressor activity, the distinction between them not being clear. The responses in the two segments were nearly in phase, the bursts overlapping in time. The metathoracic spikes began a few milliseconds earlier than the earliest mesothoracic ones.

The results show that the thoracic nervous system of this locust when stimulated continuously by way of the wind-sensitive head hairs can produce an output in the flight motor axons which resembles the basic flight pattern but at lower frequency.

The possibility for phasing feedback is limited in the most extreme preparations to muscles in the head and to non-moving structures of the head or thorax. That the response of the isolated cord system is really a flight response is suggested by several facts.

(*a*) The sensory input used to elicit the response was one which normally produced flight.

(*b*) Many of the muscles from whose axons recordings were made can move the wings only. They are normally quiet when the wings are not moving in flight (Weis-Fogh & Wilson, 1961).

(*c*) The gross pattern of firing was like that in flight.

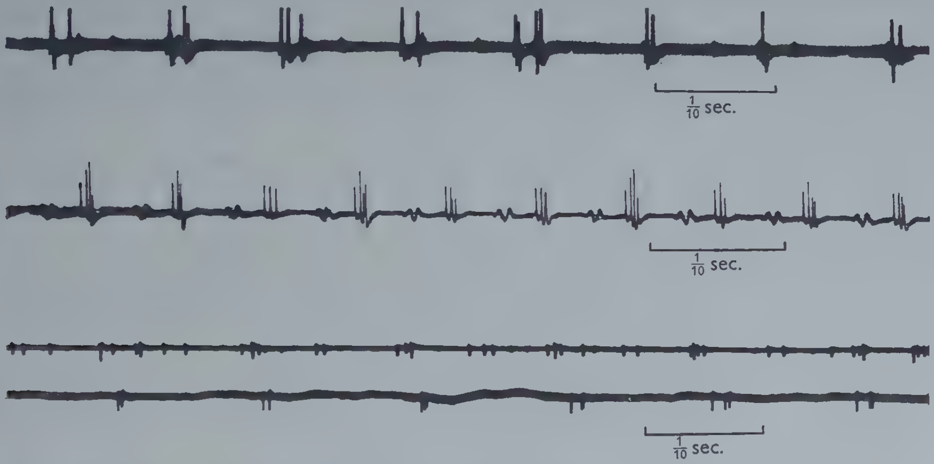

Fig. 7. First strip, discharge in the central stump of metathoracic nerve I of a head and ventral cord preparation during response to wind on head. Second strip, discharge in the central stump of mesothoracic nerve I during flight of a nearly whole animal. Third strip, discharge in the central stumps of the mesothoracic (upper trace) and metathoracic (lower trace) nerves I of a head and ventral cord preparation during response to wind on head.

(*d*) The frequency of the cycling was like that of whole flying animals in which the sensory wing nerves had been cut (and also the motor nerve for the dorsal longitudinal muscles). Further reductions in possible input sources had no further effects.

When some muscle stumps were left connected other details of the normal pattern could be recognized since more specific identification of output could be made.

V. *Responses to electrical stimulation*

Evidence for a thoracic oscillator.

Stimulation of the central nervous system anywhere from the brain to the abdominal cord could produce movements of the flight muscles. These experiments were performed on ventral half preparations. The tracheae were usually not aerated. The responses usually degenerated rapidly, but even the animals in bad condition were useful in that they gave results on parts of the whole system. Shocks to the pterothoracic ganglia caused one-to-one responses, probably due to direct effects on the motor neurons. Elsewhere response usually required several shocks and the number required was inversely related to the stimulus intensity. Apparently synapses are involved in which both temporal and spatial summation or facilitation are effective. Depending on the stimulus parameters and the condition (mainly age) of the preparation the response varied in magnitude from a single muscle unit to the whole set of fast flight muscles. In the latter cases the response was either co-ordinated or irregular.

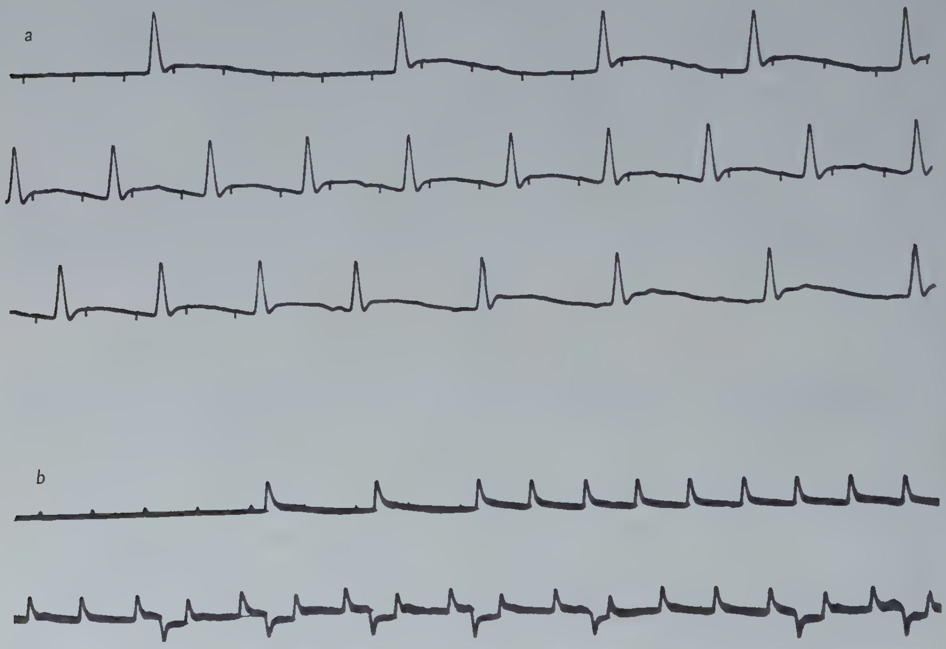

Fig. 8. (*a*) Portions of the response of one elevator muscle unit to stimulation of the anterior nerve cord. Stimulation was at 50/sec. Seventy facilitating shocks preceded the first response, sixty-four responses at exactly one-half stimulus frequency occurred before stimulation ended and fourteen after-discharges followed. (*b*) Similar to (*a*) but with the other electrode on the adjacent depressor muscle and at stimulus frequency of 25/sec.

In the case of response of only one unit the response was in phase with the stimulus but did not usually follow every stimulus (Fig. 8*a*). At stimulation frequencies between 25 and 100/sec the response followed at a fraction of the stimulus frequency which could within limits be set by intensity of the stimulus. Most often the response followed exactly every *n*th stimulus. By careful adjustment of the stimulus parameters an unstable condition could be achieved in which the response fluctuated between every *n*th and $(n+1)$th stimulus. This choosing of stimuli by the follower cell indicates an oscillation within it. This oscillation manifests itself after the end of stimulation, in many cases, by after-discharge in the form of a few to many extra firings at gradually decreasing frequency (Fig. 8*a*). By varying stimulation different units have been driven at frequencies over the range of 5–10/sec. Cyclic after-discharge following a long stimulus train sometimes lasted hundreds of cycles. In rare cases the preparation became silent immediately on cessation of stimulation and after a fraction of a second pause started again for a few cycles. Similar behaviour can be seen occasionally in intact animals when the wind is stopped. In this case there may remain some external stimulation. Such a case is recorded in Fig. 4 where only elevators are seen to respond.

In fresh preparations although the response may begin in only one unit it usually spreads to others. A limited case of such spread is shown in Fig. 8*b*. An elevator unit begins firing after many facilitating stimuli and it follows in phase with the stimulus at 25/sec. After many cycles the adjacent depressor joins the response in a nearly constant phase relationship, but not synchronous, with the elevator and at a lower rate. It appears that an intermediate driving step is involved which requires greater facilitation. This may be by way of the elevator itself. In nearly all cases of stimulation of the cord elevator muscles showed the lower threshold.

When the spread of excitation is great the response may lose its phase relationship with the stimulus, and the co-ordination between elevator and depressor muscles may also be upset. In some cases, however, the whole system of fast flight muscles is activated in what appears to be the normal co-ordination. Measurements have been made from not more than two muscles at a time, but these and visual observations reveal a high degree of co-ordination. In cases both of wind and electrical stimulation one or more elevators often began firing at an excessively high frequency but when the depressors were added a more normal frequency was established. The same phenomenon occurs at the beginning of normal flight (Fig. 4). In these more co-ordinated preparations adjacent elevator-depressor pairs were observed under a wide variety of stimulating conditions. After an ordinary train of facilitating stimuli the stimulus frequency was decreased and, if necessary, the intensity increased to find the minimum possible frequency for maintained response. At a frequency as low as 5/sec. each shock may produce an elevator followed by a depressor response. The responses of the two muscles are a few tens of milliseconds apart with a long silence until the next stimulus. If at a frequency of 5/sec. the stimulus intensity is increased greatly the response rate may jump to twice that of the stimulus frequency (or some other small whole number multiple). The pairs of responses are nearly evenly spaced and not grouped right after the stimulus. By means of high frequency, high intensity, short bursts of stimulation via preganglionic fibres the flight motor neurons were stimulated at even lower overall frequencies. When bursts of stimulation, at 300/sec. and lasting 1/10 sec. were repeated at 2/sec. a general tetanus occurred during the burst, but in the interval between there were 4 to 5 cycles of elevator depressor activity in a smooth rhythm at around 10 cycles/sec.

The above preparations lacked wing sense organs and all the responding muscles were transversely bisected. The head could be removed without effect. Stimulation anterior or posterior to the prothoracic ganglion was similar in effect and in some cases that ganglion as well was removed.

Multiplicity of oscillators and input pathways

One of the previously mentioned animals, in which the connectives and recurrent nerves between thoracic ganglia 2 and 3 were cut, was prepared for electrical stimulation. Stimulation of the anterior part had an effect similar to wind on the head. It produced rhythmic movements of the flight muscles of the mesothorax. Stimulation of the abdominal cord did the same in the metathorax, presumably through fibres from the anterior which have responded antidromically. The two could be excited simultaneously but independently. Separate oscillators must exist in the two segments.

If only one anterior connective is stimulated (behind the head) the response begins on the same side in the elevator muscles. After a few cycles the adjacent depressors begin. The contralateral muscles begin still later, but perfectly in phase. If the other connective is stimulated the sequence starts on that side.

High-frequency stimulation of the supra-oesophageal ganglion of one animal caused the two most posterior elevator muscles to fire at independent rates with no steady phase relationship. Similar cases have been observed between other muscles. Several oscillators in each segment become necessary to explain this abnormal type of behaviour. In the very worst preparations chaos results when these oscillators are not acting together. In these cases no sign of co-ordination is apparent and muscles fire at diverse rates.

Systematic study of the effects of stimulating the peripheral nerves has not been undertaken. A few observations show that muscular activity can be elicited by stimulating the sensory branches of nerve I. These reflex effects probably account for the after-discharge in Voskresenskaya's nerve-muscle preparations (Ewer & Ripley, 1953; Chadwick, 1953). In numerous cases stimulation of the stumps of the wing sensory nerves of quiet preparations caused activity in one or several flight motor units. A co-ordinated whole response was not evident. In five preparations an attempt was made by means of stimulation of these nerves during the ordinary response to wind on the head to gain control of the timing of the oscillations. The results were all negative so that no specific timing signal from the wings has been found as yet. Small changes in frequency could be produced in this way.

The frequency of discharge is affected by normal types of stimulation as well. Increasing wind speed increases frequency, but this usually adapts unless the system is near to threshold at first. Increasing light intensity, when the eyes are connected, may cause either increase or decrease in frequency. The conditions determining the sign of the reaction are unknown. This change also adapts. The effects of wind and light and of electrical stimulation of the sensory nerves are additive.

Only one case in which a mesothoracic depressor muscle fired at lower threshold than any elevator during stimulation of the anterior cord has been observed, but this case is sufficient to prove that at least some parallel paths exist from the head to the antagonistic pairs. In this case the elevator muscle responded later and as in more normal cases the two never fired together. Specific input from the head to the depressor muscles is certainly to be expected if these are the only controller muscles. Stimulating nerve I*A* can result in selective depressor responses also.

Stimulation across the supra-oesophageal ganglion of intact or dissected animals has given various fragmentary responses from firing of a single unit to massive response of the elevators like that in the beginning of flight. A means of stimulation which would keep flight going without wind has not been found although this could be expected from appropriate brain stimulation. In one case flight could be abruptly inhibited by shocks at 100/sec. As long as the stimulation continued the animal maintained a resting posture with the wings folded. Flight was resumed immediately after the stimulus was turned off.

Excitability of the peripheral motor axon

Features of the ganglionic (synaptic) transmission to the flight motor axon can be mimicked by a peripheral nerve preparation of axons to the dorsal longitudinal muscle. Ewer & Ripley (1953) found in a nerve-muscle preparation that the axons would respond to only a fraction of near-threshold stimuli. This result has been duplicated on isolated nerve under conditions in which relative refractoriness could be separated from late supernormality. Slight refractoriness can be detected for up to 100 msec. This is about 10 times as long as figures given for central axons in *Periplaneta* (Boistel, 1960). At 30 msec. the threshold was about 110% of resting threshold. The axons could not be caused to fire repetitively to single shocks applied externally, although stimulus intensities were increased to those which caused irreversible damage and durations up to 1 sec. were used.

DISCUSSION

The specific hypothesis of Weis-Fogh (1956*b*) concerning the origin of the flight co-ordination in locusts and the opinion expressed by Pringle (1957) suggest a reflex explanation of insect flight movements. The evidence presented here supports strongly the notion that there is an innate central pattern for the production of flight movements in the locust. In some of these experiments the only source of feedback from muscle activity was from head structures. In others the head was removed but portions of the thoracic muscles remained. In all cases where input was deranged there was no further effect on frequency and co-ordination beyond that produced by cutting the main sensory nerves from the wings. Either a normal flight-inducing stimulus or special electric shocks could produce a pattern of contractions like those in flight. The existence of an innate pattern does not preclude the possibility that peripheral loops are *also sufficient* to produce the same pattern, but important as those feedbacks must be for the setting of the frequency of cycling, for accurate timing of contraction cycle and movement cycle, and for fine adjustments for control manœuvres they act on top of what is already determined by the central nervous structure and function. It is possible that the inherent and the reflex mechanisms operate in parallel upon the motor neurons, but it is also possible, and seems more probable to the author, that the two operate through the same elements. Indeed, the evidence does not yet indicate internuncial integrative cells. In unco-ordinated preparations there seem to be as many pacemakers as there are motor units. The hypothesis which emerges is that many pacemakers, which could be the motor neurons themselves, are each capable of independent oscillation over a wide frequency range but that when many act at once they are coupled into a stable pattern with a single dominating frequency which is dependent on total quantity of input. Specific changes in input to a certain motor unit may change the position of that unit in the over-all pattern.

The nature of oscillation of the individual pacemakers is not clear. The similarity of the relative refractory period of the motor axon to the interval at the lower limit for rhythmic oscillation of the flight system may be only a coincidence, but it appears a possible mechanism for oscillation and at least if not of basic importance an indication that many factors are working together to provide a fool-proof system. The main argument against a refractory type or spontaneous relaxation oscillator is the fact that many, if not all, of the motor units can fire not once but twice in rapid succession during a single cycle. This double firing may be due to a particularly massive input capable of exciting even a rather refractory neuron. It has already been shown that some of the inputs do operate discontinuously and we have also found that some of the double firing must be due to specific input to certain cells since the controller depressor muscles show antagonism in this respect as part of their flight control mechanism (Weis-Fogh & Wilson, 1961). The pacemakers having no fixed frequencies, it should be possible to set not only the phase of each but also its frequency. This could be done by resetting the phase on each cycle. Transganglionic stimulus-recording experiments showed that this can occur when few units are active. Although a unit may discharge rhythmically by itself during pauses in stimulation it usually follows in a fixed phase relationship during stimulation.

Until the several pacemakers are individually better understood it is futile to discuss how they might be coupled. It is interesting, however, that if the above hypothesis is correct in principle, if not in detail, then the vague concept of a 'flight control centre' is supported neither in the anatomical sense nor in the sense of a group of hypothetical neurons which are uniquely concerned with integrating input with a certain pattern and passing this on to 'motor centres'.

The animals with reduced input never gave co-ordinated responses at a frequency greater than normal. Although all parts of the cycle slowed somewhat, the greatest part of the reduction in frequency was due to a longer interval between depressor action and elevator action. This and the fact that elevator action normally starts before depressor action in most preparations suggest that the sequence of events is an open chain or one with a very weak link, and that a triggering occurs at the metathoracic elevators. This triggering may be due to the pacemaker activity in the case of lower frequency deafferented preparations. In normal animals the higher frequency may be due only to an increased pacemaker activity because of an increased quantity of input but, after flight has started, the system may also be triggered by specific timing input. Excitatory connexions between successively acting sets of muscles seem likely. The elevators discharge at a high frequency at the beginning of flight and at a lower rate as soon as the depressors begin, suggesting an inhibitory relationship between them. Some of the preparations indicate the same thing.

Comparisons with other systems

The ventilation and spiracular movements in the same animal have been found by Miller (1960*a*, *b*) and Hoyle (1959) to be controlled in a manner quite similar to that described here. The rhythmic output comes from several autonomous centres which can be influenced by input. A certain CO_2 level (input) seems to be necessary for cycling, but the cycling is not due to build-up and release of CO_2. The rhythm of the fastest centre sets that of the others. Thus in both flight and respiration several pacemakers, each dependent on some level of input, are able to oscillate at some frequency of their own, but are ordinarily dependent on each other in such a way that a single pattern emerges. In both cases this pattern can be modified by input. That functions so different in purpose and time course are organized similarly, in a way that is not the only imaginable organization and not even considered the most probable one in flight, may indicate that there is a basic structure of the nervous system from which the two functions evolved. The copulatory movements of mantids and roaches are also under central control modified by input (Roeder, Tozian & Weiant, 1960). Here the output is spontaneous but ordinarily inhibited by higher centres. It is, however, not known to be a patterned output. A remarkably similar situation has been described for the control of certain crustacean appendages. Hughes & Wiersma (1960) found that the isolated abdominal nerve cord produces intermittent bursts in the first nerve roots grossly similar to those which are normally associated with the swimmeret beat but differing in pattern, frequency, and phasing. Proprioceptive and descending central nervous pathways influence the pattern but are not alone responsible for it. The crayfish and locust seem to differ in the degree of perfection of the central co-ordination for these activities, but there is no suggestion of different basic mechanisms.

The experiments on vertebrates, mainly of Gray & Lissmann (1946), Lissmann (1946) and Weiss (1950), have shown that some degree of innate central patterning must exist. The patterned outputs are apparently not spontaneous but depend on some input from proprioceptors. It is not decided, however, whether the pace-setting is dependent on the timing of the input or on a central pacemaker which needs only to be sufficiently excited.

The existence of proprioceptive leg reflexes (Pringle, 1940) and close observations of normal walking and the effects of amputations (Hughes, 1952, 1957) in cockroaches have led to a reflex hypothesis for locomotion here as well. Pure reflex mechanisms seem likely in some centipedes (von Holst, 1935). However, experiments which seem to demonstrate the sufficiency of feedback loops do not prove the lack of a central pattern. In the case of locust flight the mechanism could have been a pure reflex system. It seems not too early to conclude that central oscillators in arthropods are of such fundamental importance that they are used even where other mechanisms might suffice.

SUMMARY

1. The co-ordination of the flight movements of *Schistocerca gregaria* Forskål was examined in order to determine the extent of central patterning and reflex control.

2. Electrical recordings from wing sensory nerves showed many units which responded to wing movements of various kinds. During flight the sensory discharge was timed to certain phases of the wing-beat cycle.

3. Surgical removal of the sources of timed input did not abolish patterned output, which resembled that during flight, but the frequency of cycling was considerably reduced. Either electrical stimulation of the nerve cord or continuous wind on the head could elicit the pattern.

4. A multiplicity of oscillators in the flight control system was demonstrated.

5. It is suggested that the basic co-ordination of flight is an inherent function of the central nervous system but that peripheral feedback loops influence the frequency of operation and details of pattern.

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THE ROLE OF THE ANTENNAL GLAND IN THE OSMOTIC AND IONIC REGULATION OF *CARCINUS MAENAS**

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INTRODUCTION

Several previous studies on various littoral and semi-terrestrial crabs [*Carcinus maenas*—Nagel (1934), Webb (1940); *Pachygrapsus crassipes*—Prosser, Green & Chow (1955), Gross (1959), Gross & Marshall (1960); *Uca pugnax* and *U. pugilator*—Green, Harsch, Barr & Prosser (1959)] have pointed out the effective role of the antennal gland in regulating the ionic concentration of the urine in sea water of various concentrations. Recently, Flemister (1958) and Green *et al.* (1959) have suggested that in certain of the more terrestrial crabs (*Uca* spp. and *Gecarcinus lateralis*) water may be reabsorbed from the urine. Riegel (unpublished) has obtained direct evidence of the withdrawal of water from the urine of *Hemigrapsus nudus*. As will be shown here, specimens of *Carcinus maenas* are also capable of withdrawing water from their urine in 100% sea water or in water-saturated air. In addition, specimens of *C. maenas* can also alter the urine/blood (hereafter designated U/B) ratios of sodium, magnesium, chloride, osmotic pressure and possibly calcium.

MATERIALS AND METHODS

Specimens of *Carcinus maenas* were obtained from the Marine Laboratory, Plymouth, and maintained in the sea-water system of the Zoology Laboratory, University of Cambridge. They were not fed during the experiments.

Three separate sets of experiments were undertaken: (1) At 24 hr. intervals measurements were made of the weight of crabs with unblocked nephropores and of crabs with nephropores blocked with dental cement. (2) The concentration of inulin and sodium in the blood and urine was measured in two groups of crabs over periods of 4 and 5 days. Inulin was injected just prior to placing the crabs in water-saturated air. (3) The concentrations in blood and urine of inulin, sodium, potassium, calcium, magnesium, chloride and the osmotic pressure were measured in nine animals over a 4-day period. In this case inulin was injected 3 weeks before the animals were placed in air, thus ensuring that a steady state had been reached in the U/B ratios before the start of the experiment.

Urine. It was collected by lifting the nephropore flap and aspirating the emerging fluid into a glass pipette. Samples of urine were divided as soon as possible after withdrawal from the animal as follows. A 20 μl. sample for inulin determination was placed in an aluminium planchette containing 1 ml. of distilled water to which a small amount of wetting agent had been added. Two samples (by dropping-pipette, vol. *ca.* 1·5 μl.) for sodium determination were placed in vials containing 4 ml. of distilled water. Two samples (by dropping-pipette, vol. *ca.* 9 μl.) for potassium and calcium determinations were placed in vials containing 2 ml. of distilled water. Two samples (by dropping-pipette, vol. *ca.* 3·5 μl.) for calcium plus magnesium (*i.e.* total hardness) were placed in vials containing 2 ml. of distilled water. The remainder of the urine was stored under liquid paraffin, and from this samples for osmotic pressure and chloride determinations were taken.

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Blood. It was collected with the aid of a 0·25 ml. syringe from the basal portion of one of the walking legs. After collection, it was handled in the same manner as the urine. The same dropping-pipettes were used for both blood and urine. Self-levelling pipettes of 40–70 μl. capacity were used for inulin determinations on blood.

Determinations of the osmotic pressure and of the concentrations of inulin and ions in blood and urine were carried out as follows: sodium, potassium and calcium were read directly in an EEL flame photometer with corrections for interference by other ions. Chloride was estimated by the first method of Ramsay, Brown & Croghan (1955). Osmotic pressure was measured by the freezing-point method of Ramsay & Brown (1955). The inulin used in these experiments was carboxy-labelled with ^{14}C; it was determined with a conventional gas-flow Geiger counter, all samples being counted to at least 1000 counts. Magnesium and calcium were determined by the ethylenediaminetetra-acetic acid (EDTA) method of Mr A. A. Wilson (personal communication) modified for small quantities. The standard sample (*c.* 3·5 μl.) of blood or urine was placed in 2 ml. of distilled water and buffered with 1 drop of ethanolamine buffer (31 ml. ethanolamine plus 5·5 ml. conc. HCl made up to 100 ml. with distilled water). Two drops of 0·7 M-KCN and 3 drops of Solochrome Black 6 BN (0·1 % in ethyl alcohol) were added. The resulting solution was titrated with the EDTA solution (3·72 g./100 ml.) delivered from an Agla micro-burette. The colour of the solution changed from rose pink to sky blue at the end point.

RESULTS

Weight changes in water-saturated air

Average daily changes in weight for 4 and 5 days are shown in Table 1. In the first group of crabs the average daily weight change appeared to remain relatively constant throughout the 4-day period. In the second group of crabs the average daily weight loss decreased during the 5-day period. As might be expected, the over-all loss of water from the group of smaller crabs (average weight, 53·6 g. at 6 hr.) was greater than the loss from the group of larger crabs (average weight, 83·7 g. at 4 hr.).

There was no difference in weight loss between crabs whose nephropores were blocked and those whose nephropores remained unblocked.

Table 1. *Average weight loss (% of the body weight) per 24 hr. of 16 specimens of* Carcinus maenas *in water-saturated air for 4–5 days*

Group	Mean weight (g.)	24 hr.			48 hr.			72 hr.			96 hr.			120 hr.		
		Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range
I	53·2	1·25	1·20	0–3·43	1·51	0·43	0·72–2·40	1·53	0·40	0·74–2·11	1·33	0·31	0·77–1·82	—	—	—
II	83·7	1·58	0·18	0·89–2·88	1·17	0·25	0·94–1·73	1·13	0·18	0·91–1·56	—	—	—	0·85	0·10	0·82–1·06

Table 2. *Changes in the osmotic pressure and in the concentrations of various ions and of inulin in blood and urine of 9 specimens of* Carcinus maenas *placed in water-saturated air for 4 days*

(Figures in bold face are those which differed significantly ($P = < 5\%$) from the zero-time value.)

Time from start of the experiment ...	0 hr.			24 hr.			48 hr.			72 hr.			96 hr.		
	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range
Na (mM./l.)															
Urine	481	37·8	418–536	485	29·9	425–526	459	30·8	400–502	**423**	38·1	342–468	**391**	50·0	308–443
Blood	525	10·9	513–552	530	16·6	508–564	538	18·9	503–573	533	14·3	510–551	**543**	19·5	513–583
K (mM./l.)															
Urine	12·4	0·57	11·6–13·6	11·8	0·69	11·0–13·4	12·1	0·57	11·5–13·3	12·3	0·59	12·0–13·6	**13·4**	0·79	12·3–14·8
Blood	12·7	0·85	11·5–14·2	**11·5**	0·59	10·7–12·6	12·4	1·39	10·8–14·8	12·7	0·94	11·1–14·1	**13·6**	0·66	12·4–14·6
Ca (mM./l.)															
Urine	17·2	1·25	15·5–19·2	17·1	1·30	14·3–19·1	**21·3**	2·24	18·3–25·6	**19·8**	2·23	18·3–23·2	**22·3**	5·03	16·7–34·5
Blood	14·3	1·41	12·8–17·3	13·4	1·68	11·2–16·3	14·6	1·10	13·1–16·7	12·4	1·27	10·6–14·5	15·2	2·21	13·6–20·4
Mg (mM./l.)															
Urine	89·4	35·0	41·6–150	85·0	34·0	53·0–157	**121**	47·0	80·0–191	**163**	36·0	116–231	**192**	37·0	153–250
Blood	21·2	2·90	16·8–25·8	21·5	2·30	19·8–27·7	20·0	2·00	17·5–24·9	23·7	3·00	19·4–30·8	19·4	3·30	15·6–25·5
Cl (mM./l.)															
Urine	531	35·6	434–558	**577**	14·6	552–589	671†	86	594–801	**568**	18·8	533–589	**626**	14·6	597–647
Blood	502	22·5	458–532	490	20·3	458–523	**569**	26·8	521–621	543	43·8	458–560	**558**	19·5	512–585
Inulin* (% orig.)															
Urine	202	27	165–246	179	35	117–246	187	43	108–246	195	35	123–256	**211‡**	30	135–274
Blood	100	0	0	**92**	8·4	82–107	**81**	10·5	66–101	**85**	6·9	71–97	**87**	6·6	76–98
Osmotic pressure (M-NaCl)															
Urine	0·559	0·004	0·555–0·570	—	—	—	—	—	—	—	—	—	**0·646**	0·019	0·620–0·685
Blood	0·568	0·014	0·550–0·645	—	—	—	—	—	—	—	—	—	**0·622**	0·015	0·600–0·645

* Urine values as a percentage of blood at 0 hr. † This value unreliable. ‡ Significantly different from the figure for 24 hr.

Ion concentrations in blood and urine

The results of determinations of sodium concentration in blood and urine of uninjected crabs living under 'normal' conditions in the sea-water aquaria are shown in Fig. 2. In these animals there was considerable variability of sodium concentration in both blood and urine.

In Fig. 1 are shown the U/B ratios of sodium and inulin for crabs injected with inulin imediately prior to being placed in water-saturated air. There was a steady and significant decline in the average sodium U/B ratio, while the average inulin U/B ratio rose throughout the experiment.

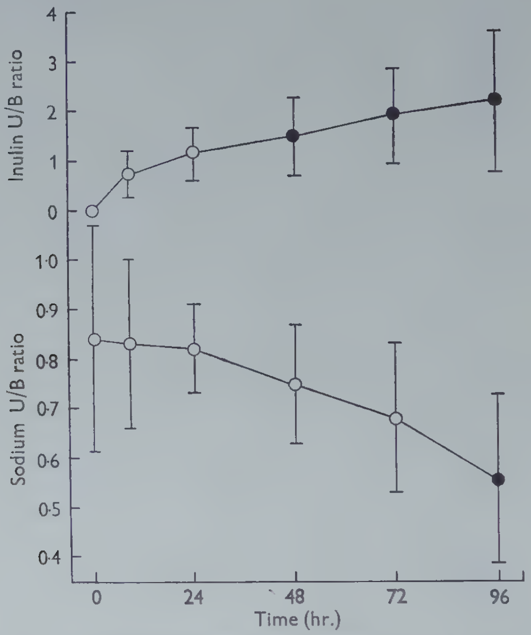

Fig. 1. U/B ratios (mean and standard deviation) of sodium and inulin for 14 specimens of *Carcinus maenas* in water-saturated air for 4 days. The sodium U/B ratio at zero time is the average for animals living in the sea-water aquarium. Open circles denote no significant difference ($P = > 5\%$) from the 8 hr. U/B ratio. Closed circles denote a significant ($P = < 5\%$) difference from the 8 hr. U/B ratio.

In Table 2 are shown the average values, for urine and blood, of the concentrations of sodium, potassium, calcium, magnesium, chloride, inulin and of osmotic pressure, before exposure to water-saturated air and after 4 days of exposure to that environment. In this experiment the inulin was injected 21 days prior to placing the animals in water-saturated air. Significant changes in the potassium concentrations were seen in the blood at 24 and 96 hr. and in the urine at 96 hr. The calcium and magnesium concentrations of the blood did not change significantly over 4 days, whereas in the urine the concentrations of these two ions changed markedly during the experimental period. The chloride concentration and the osmotic pressure of both blood and urine showed significant increases from the zero-time values. The osmotic pressure of the urine increased at a greater rate than that of the blood, so that the osmotic pressure of the urine was significantly in excess of that of the blood after 4 days. The sodium concentration of the blood increased gradually, but there was a marked decrease in the sodium concentration of the urine. Curiously, there was a decline in the inulin concentration of both blood and urine between zero time and 24–48 hr., with a subsequent gradual rise in those values throughout the remainder of the experiment. Because of the decline in the inulin concentration of the urine no significance could be attached to differences in the concentrations of that substance in the urine between zero time and 24, 48, 72 and 96 hr. However, a test of significance between the 24 and 96 hr. values for inulin concentration in the urine showed that these were significantly different.

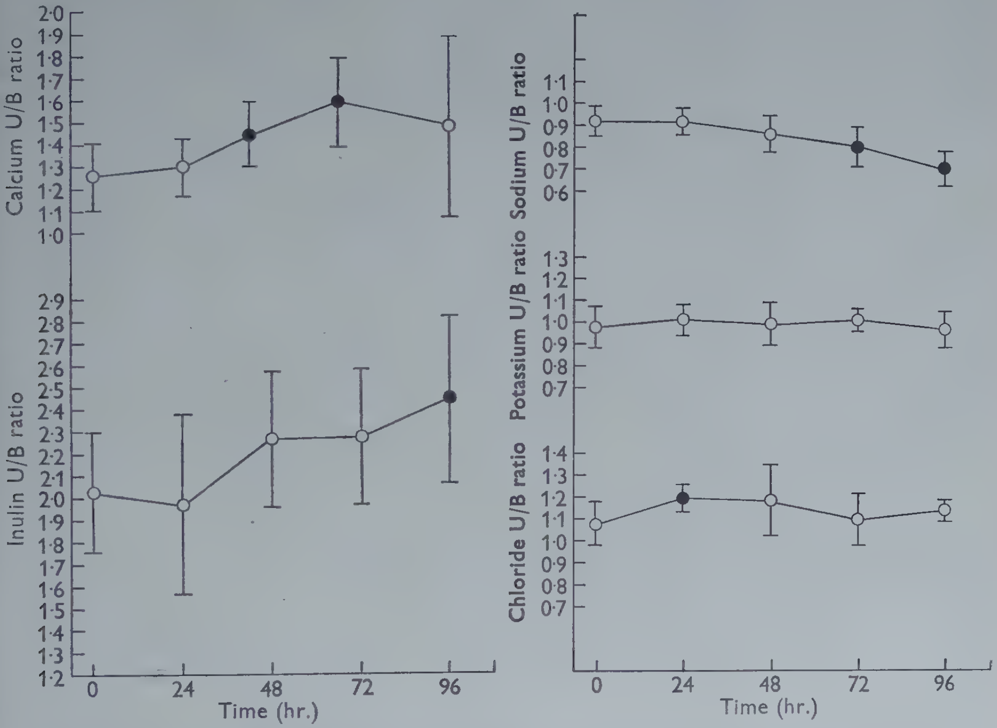

Fig. 2*a*. U/B ratios (mean and standard deviation) of calcium, inulin, sodium, potassium and chloride for 9 specimens of *Carcinus maenas* placed in water-saturated air for 4 days. Open circles denote no significant difference ($P = > 5\%$) from the zero-hour value. Closed circles denote significant differences ($P = < 5\%$) from the zero-hour value.

Figs. 2*a*, *b* shows the U/B ratios for the various ions measured and for inulin. The U/B ratios for neither potassium nor chloride showed significant changes over the 4-day period. The U/B ratios for calcium at 48 and 72 hr. were highly significantly different from the zero-time values, but inexplicably dropped at 96 hr. After 48 hr. the U/B ratios for magnesium and sodium showed characteristic and highly significant changes as compared with the zero-time values.

DISCUSSION

In the following discussion the assumption will be made that a 'filtration' process is responsible for primary urine formation in specimens of *Carcinus maenas*. 'Filtration' implies only that the formation of urine involves the passive movement of fluid into the lumen of the antennal gland, but it does not necessarily imply that the force responsible for the passive movement is hydrostatic pressure [see Riegel & Kirschner (1960) for a fuller discussion of this problem].

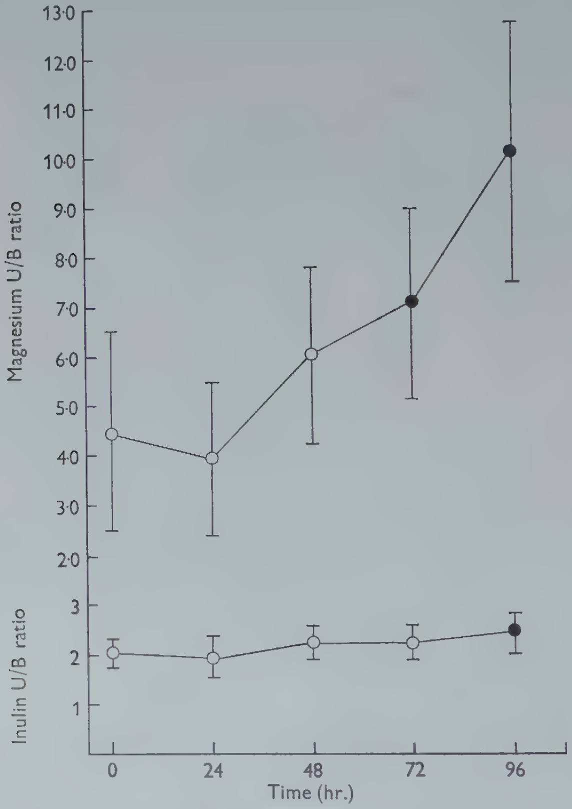

Fig. 2*b*. U/B ratios (mean and standard deviation) of inulin and magnesium for 9 specimens of *Carcinus maenas* placed in water-saturated air for 4 days. The significance of the means denoted as in Fig. 2*a*.

Water balance

The data presented in Figs. 1 and 2 *a*, *b* indicate that water is reabsorbed from the urine. In almost all cases the inulin levels in the urine exceeded those in the blood after 72 hr. in the experiment summarized in Fig. 1, and throughout the experiment summarized in Figs. 2 *a*, *b*. Fig. 1 also shows that urine formation undoubtedly was continuous throughout the experiments, although it gives no information as to possible changes in the rate of urine formation. In the group of crabs which had been injected with inulin 3 weeks prior to exposure to water-saturated air the average initial inulin U/B ratio was *ca.* 2. It seems probable, therefore, that in normal sea water there is also considerable withdrawal of water from the urine.

Evidence which has been presented previously can be interpreted to indicate that water is reabsorbed from the urine of certain crabs. Nagel (1934) has shown that in 11 out of 14 specimens of *C. maenas* injected iodine was more concentrated in the urine than in the blood after the crabs had been left in air for 10 hr. Nagel interpreted his results as indicating the active secretion of iodine into the urine, but alternatively they could indicate water reabsorption. Flemister (1958) has shown that the land crab, *Gecarcinus lateralis*, reabsorbs water from its urine when placed in dry conditions. Flemister stated that water reabsorption was shown by the fact that the concentration of chloride and SCN in the blood remained constant after 24 hr., while the inulin concentration fell. Much less equivocal evidence for water reabsorption from the urine of *G. lateralis* was given by the fact that the concentration of inulin in the blood fell at all; that is, if inulin continued to be filtered by land crabs out of water its concentration in the blood would not fall unless some extra-vascular water (not containing inulin) was continually entering the blood. The most obvious source of this water would seem to be the urine. Direct evidence for the reabsorption of water in a semi-terrestrial crab was obtained from experiments carried out by one of the present authors (J.A.R.) at the Friday Harbor Laboratories of the University of Washington.* Specimens of *Hemigrapsus nudus* were injected with ^{14}C-inulin and placed in jars containing 50, 100 and 150% sea water. The jars were so filled that the crabs could not escape from the medium but could obtain air. After 24 hr. the average inulin U/B ratios were 1·33, 1·51, and 0·92, respectively, for crabs in 150, 100 and 50% sea water. For a second 24 hr. period, the crabs were permitted to climb out of the medium (which most of them did). During this time the average U/B ratios were 1·63, 1·22 and 1·41, respectively, for crabs in 150, 100 and 50% sea water. These results indicated that the reabsorption of water from the urine is a normal process in specimens of *H. nudus* when placed in air or in 100% or 150% sea water.

Concentrations of ions in blood and urine

The marked changes observed in the sodium and magnesium concentrations in the urine of *Carcinus maenas* parallel those seen in *Pachygrapsus crassipes* when desiccated (Gross, 1959) and when placed in 100 and 170% sea water (Prosser *et al.* 1955). They also parallel the values for *Uca pugnax* and *U. pugilator* in 100 and 175% sea water (Green *et al.* 1959) and agree with the previously determined concentrations of those ions in the urine of *Carcinus maenas* (Webb, 1940).

From the fact that the urine concentrations of potassium, calcium and possibly chloride did not increase to the extent which would be predicted from the inulin U/B ratios it may be deduced that those ions were withdrawn from the urine with the water. In this connexion, it is interesting to note that the two ions, calcium and magnesium, whose concentrations did not increase significantly in the blood at 96 hr. were those which showed the greatest increase in the urine throughout the experimental period. This observation suggests that the renal epithelium is relatively impermeable to those ions. While there is no evidence to indicate that withdrawal of sodium and of water are linked, it may be seen in Figs. 1 and 2*a* that the greater the decline in urine sodium concentration, the greater the rise of the inulin U/B ratio.

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* Gratitude is expressed to Dr Robert L. Fernald, Director, for providing equipment and laboratory space necessary for this study.

The magnesium concentration in the urine rose markedly during the course of the experiment; since the magnesium U/B ratios exceeded that for inulin, the high concentration of magnesium in the urine could not have been brought about solely by the withdrawal of water from the urine. It follows, therefore, that magnesium is probably secreted into the urine.

The urine and blood were close to being isosmotic and the observed increase in the urine magnesium must have been at the expense of another cation. Initially, sodium was the major cation in the urine, and it is probable that the rise in urine magnesium was due (at least partially) to the withdrawal of sodium. As seen in Table 2, the fall in the sodium concentration of the urine paralleled the rise in the magnesium concentration. However, on the basis of electro-chemical balance, the fall in the urine concentration of the monovalent sodium was insufficient to account for the rise in concentration of the divalent magnesium, in terms of a direct sodium-magnesium exchange. Magnesium salts tend to be less fully ionized in high concentrations than sodium salts, and it is possible that the actual osmotic pressure of the urine might be lower than that calculated from the sum of the ions known to be present. In order to test this a solution whose ionic composition approximated that of the 96 hr. urine was made up, containing 400 mM./l. NaCl, 112 mM./l. $MgCl_2$ and 102 mM./l. $MgSO_4$. In this solution, as compared with urine at 96 hr., the sodium concentration was raised to compensate for the absence of potassium, and magnesium, likewise for calcium. Further, it was assumed that all the non-chloride anion in the 96 hr. urine was sulphate. The osmotic pressure of this solution was equivalent to 0·615 M./l. NaCl. It seems likely, therefore, that the observed osmotic pressure of 0·645 M/l. NaCl for the 96 hr. urine is higher than that expected from the measured concentrations of the ions. This indicates the presence of other undetermined ions and metabolites in the urine. From the work of Green *et al.* (1959), it is known that large amounts of ammonium are present in the urine of *Uca pugilator* and *U. pugnax*, especially when they are placed in 175 % sea water.

The measured ionic composition of the urine of *Carcinus maenas* in water-saturated air most closely resembles that of other crabs when they are in sea water of more than normal concentration. It is likely that the problem of obtaining water is much the same to hypo-osmotic regulators in such sea-water concentrations as it is to *C. maenas* in air.

Gross & Marshall (1960) has presented evidence supporting his conclusion that the concentration of magnesium in the urine of *Pachygrapsus crassipes* bore no direct relationship to the external magnesium concentration, but was determined by the direction and magnitude of the water flux through the crabs. The present results give no unequivocal evidence linking water flux and magnesium concentration in the urine, but certainly, the marked rise in magnesium concentration in the urine was accompanied by (among other things) indications of increased water reabsorption from the urine. In turn, the increased water reabsorption from the urine presumably was due to a water stress imposed by evaporative water loss.

RESULTS

1. *Carcinus maenas* in water-saturated air lost weight at a rate somewhat exceeding 1 % of the body weight in 24 hr. Small crabs lost weight at a greater rate than large crabs.

2. Urine was formed throughout the 4-day period in air, and from this urine water was reabsorbed. There was evidence that water reabsorption from the urine also took place in crabs kept in normal sea water.

3. The osmotic pressure of urine and blood rose during the exposure to air, and after 4 days the urine osmotic pressure was significantly in excess of that of the blood. The elevation of the osmotic pressure was probably a consequence of the loss of water by evaporation.

4. The rise in blood osmotic pressure was accompanied by significant elevations (after 4 days) of the concentrations of chloride, sodium, potassium and inulin. The calcium and magnesium concentrations in the blood did not change significantly over the 4-day period.

5. The rise in urine osmotic pressure was accompanied by significant elevations in the concentrations of chloride, magnesium, potassium and calcium in the urine. The sodium concentration fell significantly.

6. It is suggested that water withdrawn from the urine was accompanied by all ions measured, except magnesium, which was possibly actively secreted into the urine.

7. The results of the present study were very similar to those of other studies on semi-terrestrial and littoral crabs kept in sea water of normal concentration or above.

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